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The Biochemistry of Benzpyrene*

I. A Survey, and New Methods of Analysis

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INTRODUCTION

DEVELOPMENT OF KNOWLEDGE ON THE METABOLISM OF 3,4-BENZPYRENE

The fate of benzpyrene after administration to animals was first investigated by Peacock (9, 20, 21). By making use of the characteristic fluorescence spectrum he was able to discover the main facts: that it is removed from the blood stream in unaltered form by the adipose tissue and all tissues and glands containing fat solvents, and especially that it is metabolized to new derivatives having different fluorescence spectra. These researches were continued in Glasgow by Peacock in co-operation with Beck, Chalmers, and Kirby; at Oxford by Berenblum, Crowfoot, Holiday, and Schoental; and at the Mount Vernon Hospital in Northwood by Doniach, Mottram, and Weigert. The following is a list of the facts so far established:

1. The course of events is always essentially the same and independent of the mode of administration, whether by intravenous, subcutaneous, intraperitoneal, or intramuscular inoculation; by feeding, or by painting the skin (11, 12, 20, 21).

2. Goats, rats, mice, and fowls show essentially the same mechanism of elimination (20), whereas in rabbits there are indications of a different metabolism (4).

3. Unchanged hydrocarbon disappears completely from the site of application after varying periods of time, and very little appears in the unchanged form in the feces, urine, or other secretions (3, 5, 6, 8-10); only after feeding (12) do considerable amounts of unchanged hydrocarbon appear in the feces.

4. The most conspicuous event is a chemical change of benzpyrene, indicated by disappearance from the fluorescence spectrum of characteristic violet bands, which are replaced by bands in different positions. The substances giving rise to this new fluorescence spectrum have been called "BPX" when found in the bile and intestinal contents (1, 2, 4-7, 21-24), and "tissue-BP-blue" when found in the liver, kidney, lung, mammary glands, subcutaneous tissues, and skin (11, 12).

5. The substances are produced locally at the sites where they appear; *i.e.*, in the liver (for bile), kidney, lung, and mammary glands from benzpyrene transported by the blood stream; in the subcutaneous tissue from implanted, and on the skin from painted, hydrocarbon. In the kidney they are confined to the cortex; and in the skin, to the cells of the malpighian layer (11, 12, 19).

6. The BPX of the bile on entering the intestine is partly adsorbed to the walls of the small intestine and partly passes into the cecum, where it is converted into a new substance, "BPF," having a new fluorescence spectrum (5, 6, 12, 21, 22, 23, 24); this is excreted in the feces together with traces of BPX and of unchanged benzpyrene (8).

7. BPF has been found only in the contents of the large intestine; never in fresh tissues, except occasionally in the lungs (11, 12, 22, 24).

8. In all tissues that are not quite fresh the blue fluorescence characteristic of BPX and tissue-BP-blue soon changes to the blue-green fluorescence characteristic of BPF. This change, which occurs quickly at raised temperature, is inhibited by storage of the tissues in formol (22, 24).

9. The urine contains only very small amounts of either changed or unchanged benzpyrene (5, 8, 20, 21).

* This work was aided by a grant from the British Empire Cancer Campaign. A preliminary note has already been published (23).

† Dr. Mottram died on October 4, 1945.

10. The wall of the small intestine is readily permeable to benzpyrene, but not to BPX (12).

11. Normally no changed benzpyrene appears in the blood stream, but when bile is prevented from entering the intestine by ligature of the common bile duct, PBX enters the blood stream with the bile and becomes fixed to the wall of the digestive tract, stomach, and the small and large intestines (12).

12. From extractions of the feces containing BPF, crystals of monohydroxy-benzpyrene have been isolated (7), having the hydroxyl group at its 8-position (1, 2, 4).

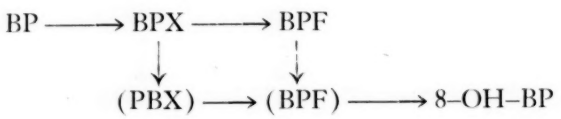
13. The absorption spectrum of BPX is quite different from that of 8-hydroxy-benzpyrene (22, 24).

14. The alumina chromatograms of extracts of BPX and BPF show the presence of a number of derivatives with similar but not identical properties (22, 24).

15. In tissues containing BPX and BPF, 3,4-benzpyrene-5,8-quinone appears in increasing amounts after death (1, 2, 4).

NEW EXPERIMENTS

The compilation in the preceding paragraphs gives the qualitative evidence that benzpyrene is transformed into various metabolites at a number of sites in the living animal. The next problems to be attacked were the chemical constitution of the various products of the metabolic conversion, the establishment of their mutual relationships, the physical chemistry of the metabolism, and the biological activity of the metabolites. The second of these found a provisional solution (Introduction: ¶¶ 6-8) that was based on observation of the spectra of the fluorescent compounds before and after their extraction from the tissues, and led to the formulation of the following tentative scheme (22, 24):—



In this scheme BPX and BPF stand for the fluorescent compounds in the respective tissues, and (BPX) and (BPF) for the substances purified by chromatography that could be extracted from the fluorescent tissues and characterized by their absorption spectra. The BPX compounds are, as shown, precursors of the BPF compounds, and these transformations occur *in vitro* after the death of the animal, whereas the production of BPX from benzpyrene occurs only in the living tissues. The evidence that the metabolic conversion of benzpyrene occurs in various steps and, in addition, some qualitative observations on the behavior of the extracted compounds, justified resumption of the study on a much larger scale in order to establish the finer details of the metabolism. A brief account of the results has been published recently (23). It was shown that there appear in the course of the metabolism a number of benzpyrene derivatives related to BPX and BPF; they were termed X_1, X_2, F_1, and F_2 respectively, and their mutual relationships and optical and chemical properties were investigated.

A. Administration of Benzpyrene to Experimental Animals

The introduction of benzpyrene into animals can be done by any of the six methods mentioned in the Introduction under paragraph 1. In order to ensure a high content of X_1 or X_2 in the tissues it is important to administer large amounts of the hydrocarbon quickly, which can be easily achieved by intravenous injection of a concentrated and finely dispersed colloidal preparation in mice. Under these conditions the maximum amount of X_1 in the small intestine, which is the best source, is reached after about $1\frac{1}{2}$ hr.; later it disappears again when it is transformed into F_1 and F_2 after its passage through the ileocecal valve.

The finely dispersed colloid was made by mixing 1 volume of a 0.1 per cent solution of benzpyrene in acetone with 2 volumes of distilled water and quickly evaporating off the acetone under reduced pressure. It then contained 0.05 per cent of the hydrocarbon. Mice tolerated very well the inoculation of 1 cc. of this finely dispersed preparation; a great advantage, since it was found that colloids prepared in the presence of NaCl coagulated quickly and gave rise to a blocking of the lung capillaries and a slowing down of the metabolism.

The best method for the production of the F-metabolites in the feces of mice is intraperitoneal injection of 1 cc. of a 0.5 per cent solution of benzpyrene in sesame oil.

B. Qualitative Analysis of the Metabolites

The chemical and physical properties of X_1, X_2, F_1, and F_2 are so different from each other, that these compounds cannot be extracted by a general method without undue loss. These differences account for some discrepancies between the results obtained from separations by our present and earlier methods. For instance, the fact that the X-derivatives escaped isolation for some time is probably due to their instability in acid media, which, on the other hand, favor the stability of the F-derivatives.

The choice of a method of preparation depends, therefore, on the purpose of a particular experiment. If it is desired to make a qualitative study of all metabolites in a tissue under definite conditions, extraction with acetone and a quick working up of the extract will give information, but complete extraction cannot be obtained by this method. On the other

hand, almost complete recovery of a particular metabolite can be achieved if the simultaneous decomposition of others is unimportant. Another experimental restriction connected with the instability of the metabolites is the time factor (Introduction: ¶¶ 7, 8, and 15). In order to avoid postmortem effects, all the operations after killing the animal must be done quickly and at low temperature.

On this account the mouse is a favorable animal, since it can be quickly dissected and small amounts of tissue transferred to nonaqueous media, dried, and purified with little loss. Since large amounts of material are not available microchemical methods must be employed for analysis, and special spectographic arrangements for recording fluorescence and absorption spectra had to be devised.

I. EXTRACTION OF THE SPECIMEN

(*a*) *Tissues.*—The following operations are with advantage carried out under an "Osira" (G. E. C.) or "Mercra" (British Thomson-Houston) mercury vapor lamp (125 W) having a Wood's glass bulb. The tissues immediately after separation are placed on ice and superficially washed with ice water. The violet-fluorescent fat is removed, and the gall bladder separated from the liver. The digestive tract is divided into stomach, small intestine, and large intestine (cecum and colon). The stomach never contains any metabolite except under the artificial conditions mentioned in the Introduction: ¶ 11. The fluorescence of the various tissues is now inspected, particularly to see how the blue fluorescence is distributed in the small intestine and to ascertain whether there is any blue-green fluorescence of F_1 or F_2 in the cecum or in the feces present in the colon.

(*i*) *Extraction with acetone.*—The tissues are quickly minced, placed in small bottles, covered with acetone,[1] and then mechanically shaken for an hour. By these means the main amounts of the metabolites are extracted from the tissues of liver, lung, and large intestine, but not from those of the gall bladder, the small intestine, or kidneys, which often still fluoresce strongly blue. Second and third extractions with acetone are often required, and these solutions are added to the first. The separation of the acetone extract is effected by centrifuging. The crude extracts usually fluoresce more or less strongly blue, and are often sufficiently colorless to allow of an approximate quantitative estimation by absorption spectroscopy of the X- and F-derivatives (see *Quantitative estimation of the various compounds*).

The separation of the various metabolites is based on the physical and chemical properties of the compounds, and includes transfers from one solvent to another, extractions, and chromatographic operations in ultraviolet illumination. This rather complex scheme (Chart I), described in detail in the text, replaces the less precise operations recommended previously (24).

All operations must be carried out at room temperature except the transfer from one solvent to another with higher boiling point under reduced pressure, which must be done as quickly as possible, and the temperature of 40° C. must not be exceeded. In avoiding unnecessarily long exposure of the extracts to even that slightly raised temperature, special vessels proved very convenient; various sizes were used in accordance with the amount of the extracts (Fig. 1). Their essential part is a measuring side-tube of 20 cc. capacity roughly calibrated in cc., a perforated stopper, and a horizontal tube for connection to an efficient pump. The acetone extract from the tissue is filled into the vessel and a known amount of xylene or other high-boiling solvent added. The acetone is evaporated at elevated temperature not exceeding 40° C. until the remaining liquid measured in the side-tube corresponds approximately to the amount of added xylene plus the water from the tissue. The turbid mixture is then emptied into a centrifuge tube, the hydrocarbon and water phases are separated, each is washed again with a few cc. of the other liquid, again separated, and the second xylene extract added to the first; the two aqueous phases are likewise combined. Usually both phases fluoresce blue because the X-derivatives are soluble in water. The separation of the various compounds starts at (I) in the Scheme (Chart I).

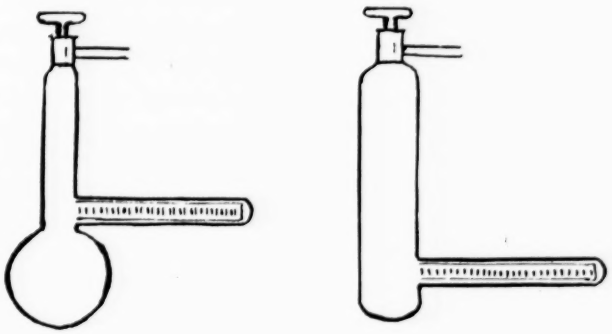


FIG. 1.—Evaporation vessel for transferring tissue extracts from one solvent to another of higher boiling point at reduced pressure.

(*ii*) *Preparation of X-Derivatives by Alcoholic Alkaline Hydrolysis:* (*a*) *From small intestine.*—In order to effect the complete recovery of the X-derivatives from the small intestine of a mouse, either immediately after its dissection or after a preliminary incomplete extraction with acetone, the entire small gut must be

[1] After this paper was submitted it was discovered that more complete extraction could be achieved with 70 per cent acetone.

hydrolyzed in a mixture of 1 to 2 cc. of saturated aqueous KOH with 9 cc. of pure methyl alcohol. About 15 minutes' boiling under reflux, or 24 hours' contact at room temperature with frequent shaking, is sufficient for almost complete lysis. Longer boiling must be avoided, even if some solid fragments remain. The mixture is cooled and cold saturated ammonium chloride (5 cc. for every 1 cc. of saturated KOH) added in order to reduce the pH to about 8. Any solid sediment is spun down in the centrifuge, the clear supernatant mixture poured into an evaporation vessel (Fig. 1), and the methyl alcohol removed by evaporation at reduced pressure in about 10 minutes. To avoid frothing, about 0.5 cc. of petroleum ether (boiling above 120° C.) is added.[2] The aqueous phase is then returned to the sediment with crystals of ammonium chloride added in excess, and extracted with 5 cc. benzene. The benzene extract contains unchanged benzpyrene, X_1, X_2, and some F-derivatives that have escaped decomposition during the lysis of the tissue. It is dried with anhydrous sodium sulphate and passed through a column of silica according to (II) in Chart I, which adsorbs the X-derivatives at the top. The filtrate contains the F-derivatives and benzpyrene, which may be separated according to (III) in the Scheme. After the extraction with benzene the aqueous phase is separated and extracted once or twice with amyl alcohol, which extracts the main amount of the X-derivatives. The extract is left in a wide beaker in a vacuum desiccator over calcium chloride for 1 to 2 days, after which the separation of the X-derivatives is completed according to (IV) in Chart I.

(β) *Preparation of X-derivatives from painted skin.*—It is not possible to extract with acetone any derivatives present in the skin, even after having cut the skin into very thin slices with a freezing microtome. Therefore the skin must be hydrolyzed with methyl alcoholic potassium hydroxide. However, since the painted skin usually contains a great excess of loosely attached benzpyrene and but small amounts of strongly fixed, unchanged hydrocarbon and derivatives, it is of advantage first to wash detached pieces with frequent changes of benzene until this no longer fluoresces. The benzene wash is then pooled to estimate the amount of unchanged, loosely attached benzpyrene by fluorescence or absorption spectrophotometry [see Section (c) below]. A washed piece of skin is left in cold methyl alcoholic potassium hydroxide (1 cc. KOH per 2 sq. cm. skin +5 times the volume of methyl alcohol) for one day and the residue reduced to small pieces with a glass rod. After treatment with ammonium chloride and evaporation of the methyl alcohol as in (α) the aqueous phase is extracted with amyl alcohol and the X-derivatives are separated according to (IV) in the Scheme. If fixed benzpyrene was present it appears in the filtrate of operation 4c in Chart I.

(γ) *Preparation from other tissues.*—Alcoholic alkaline hydrolysis is sometimes justified with tissues fluorescing blue after subcutaneous inoculation of benzpyrene, and with the small intestine, kidneys, and gall bladder of mice, rats, and rabbits. It is of advantage to separate before the hydrolysis the cortex of rabbit kidneys, which alone shows the blue fluorescence under ultraviolet illumination. Adequate amounts of potassium hydroxide diluted 5 to 10 times with methyl alcohol corresponding to the weight of the tissues must be added, but it is advisable not to exceed 20 cc. of alcohol in order to avoid an undue extension of the time of boiling during hydrolysis. Therefore it is of advantage to treat larger organs such as, for instance, the small gut of rats and rabbits, in several portions.

(*b*) *Preparation of F_1 and F_2 from Mouse or Rat Feces.*—In order to limit the secondary oxidation of the F-derivatives to benzpyrene-5,8-quinone, the feces must be placed immediately in acetone and reduced to small pieces with a glass rod under the extracting liquid. Usually only those feces passed during the first 1 or 2 days after intravenous or intraperitoneal inoculation of benzpyrene contain F_1. Two or three extractions at room temperature are usually sufficient, as can be seen by the decrease in intensity of the blue fluorescence of the successive extracts. The amount of X-derivatives and unchanged benzpyrene in the feces is usually so small that estimation of the F-derivatives by absorption spectrophotometry may be carried out with this first crude extract (see *Quantitative estimation of the various compounds,* page 106). For purification the acetone extract is transferred to xylene and the F-derivatives are extracted with 1 to 2 *N* NaOH and treated according to (III) in Chart I. F_2 is thus prepared in good yield. F_1 is better prepared from X_1 as described in footnote [4] on page 106.

(*c*) *Estimation of unchanged benzpyrene in the carcass.*—After having removed by dissection the various X- and F-bearing organs, the carcass of the mouse is hydrolyzed after the method of Berenblum and Schoental (3), with two minor changes. Instead of 50 cc. of benzene for the extraction of the hydrocarbon only 30 cc. are used, including the benzpyrene-containing filtrates collected during the separation of the various metabolites, if the intensity of their violet fluorescence justifies their being retained. Until these extracts are ready, the carcass remains in alcoholic potassium hydroxide at room temperature. Instead of estimation by fluorescence photometry we found it preferable to use absorption spectrophotometry because the

[2] This suggestion was made by Mr. Neil R. Fisk.

absorption spectra are not liable to errors from quenching of the fluorescence intensity by oxygen.

2. PHYSICAL METHODS

(*a*) *Fluorescence Spectrograms.*—A high-aperture Hilger glass spectrograph, lent by courtesy of Professor C. K. Ingold and Dr. C. F. Goodeve of University College, London, for $3\frac{1}{4} \times 4\frac{1}{4}$ inch plates was used. Throughout the whole study Kodak P. 800 plates (Supersensitive, Panchromatic, backed) were used because it was found that they did not show deceptive sensitization bands in the visible spectrum up to 640mμ. They were cut into strips one-half or one-quarter their width, which allowed 20 and 10 spectra respectively of 2 mm. width to be made on each. With the effective illumination device already described (11) the fluorescence spectra could be recorded within a few seconds to a few minutes, according to the fluorescence intensity. The principle of auto-collimation in this device allowed the fluorescence spectra of adsorbates in the chromatogram as well as of solutions to be recorded. This quick working was essential because almost all stages of the operation with more or less unstable specimens had to be recorded spectrographically.

(*b*) *Absorption Spectrograms.*—A Barfit intermediate quartz spectrograph with Spekker ultraviolet photometer by Adam Hilger, loaned by courtesy of Messrs. Adam Hilger, Ltd., London, for 5×7 inch plates was used. Because we were chiefly interested in absorption bands from 300 mμ to the visible spectrum, the plateholder was fitted with an adapter for plates $4\frac{1}{4}$ inches in length (Kodak P. 1200 plates, Super Panchro-Press). In order to identify narrow absorption bands in the spectrogram itself against a continuous background a low-voltage, single-filament, gas-filled "Osram" lamp was used. For the qualitative recording of bands the filament was projected by a quartz lens of 10 cm. focus onto the slit of the spectrograph; the formation of a sharp image of the coils of the filament in the spectra was avoided by swinging a long-focus quartz lens by means of an electric vibrator between the projecting lens and the slit. Quite uniform spectrograms extending to about 290 mμ were produced, the densities of which were measured with a non-recording photoelectric photometer (built with funds kindly presented by Messrs. Townson and Mercer, Ltd., Croydon). The sample obtainable from one mouse often did not exceed a few cc., and these small amounts were difficult to spectrograph. Since the absorption was weak in most cases, capillaries of 4 mm. diameter with quartz end-plates were substituted for the customary wide absorption cells in order to get absorbing lengths up to 20 cm. from small samples. With the quartz lens the light could easily be projected onto the slit through the whole length of the capillaries; reflections at the inner wall were avoided by stops at the entrance.

This arrangement was very suitable when only the position of the bands had to be recorded, which was done by a series of spectra with graded exposure. The aspect of such a series was similar to the quite continuous shadow-graph that can be produced by the arrangement of Holiday (14) with a logarithmic movement of the plateholder of the spectrograph during exposure.

However, the use of the capillaries in the Spekker photometer made an additional device necessary in order to obtain quantitative extinction curves of the various compounds. Because the capillary with the solution in the upper beam of the Spekker acts as a

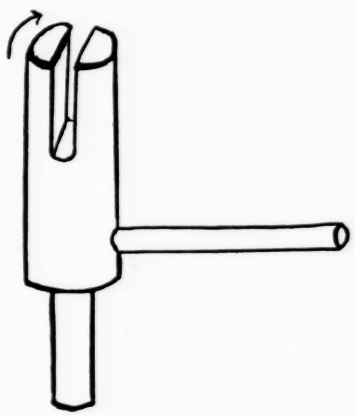


FIG. 2.—Adjustable vertical slit for the lower beam of the Spekker photometer to compensate for the reduction in the light intensity by the capillary in the upper beam.

diaphragm with the effect of an over-all reduction of intensity in the spectra, an adjustable compensating diaphragm has to be placed in the lower beam.

Near the horizontal slit of the photometer, which is controlled by the measuring drum, was arranged a vertical slit; a wide slit was cut in a vertical rod of 1.2 cm. diameter (Fig. 2), and this could be turned around a vertical axis with a lever. The correct angle and apparent width of the compensator slit could be found by examining, with the Spekker drum at zero and the spectrograph slit opened wide, the double spectrum on a ground-glass plate in the focal plane of the spectrograph. If the capillary with the colorless solution is placed in the upper beam of the Spekker and a normal wide cell with the solvent in the lower beam, the compensator slit has approximately the right width if the red ends of the two adjacent spectra are of the same length on the ground-glass plate. This must be confirmed on the finished spectrogram, and the exact position of the Spekker slit where this coincidence occurs is taken as the zero point for the extinction. The arrangement allows of the measurement of

the extinction spectra of very small amounts of absorbing compounds. For instance, 0.00045 mgm. of benzpyrene in 1.5 cc. of benzene in a capillary of 10 cm. length corresponds to an extinction of 0.5 at 385 mμ, and can be estimated without difficulty.

(*c*) *Chromatography.*—As adsorbents were used alumina (British Drug Houses, "for chromatographic adsorption analysis"); and silica, prepared after the method of Gordon, Martin, and Synge (13). The fraction of 100/150 mesh was found most suitable. Silica must be stored in a vacuum desiccator over calcium chloride.

The solvents employed for preparing the solutions and for development were benzene, xylene, amyl alcohol, and petroleum ether of boiling range 40° to 60° C. (petroleum boiling above 120° C. was used for transfers); the eluents were methyl alcohol, ethyl alcohol, and water. The compounds as first separated were usually not spectrographically pure, and required purification by repeated chromatography on silica after having been transferred from the alcohol eluates to xylene (for the X-group) or to petroleum ether boiling above 120° C. (for the F-group).

Small chromatographic tubes were generally employed, of internal diameter 8 mm. and length 15 to 25 cm., and 3 to 5 cm. of adsorbent was packed in.

3. SEPARATION OF X_1, X_2, F_1, AND F_2

(*a*) *Fluorescence Phenomena During Chromatography.*—The following fluorescence colors and spectra of the various zones on the chromatographic column, of their eluates, and of the filtrates are the guiding phenomena through the whole operation.

Adsorption on alumina from aromatic hydrocarbons.—When X_1, X_2, F_1, and F_2 are present there is seen a bluish-white top zone, which changes to blue on washing with the solvent. X_1, X_2, and F_1 are adsorbed at the surface, and cannot be separated with aromatic hydrocarbons. In the absence of the X-derivatives F_1 is adsorbed immediately under the surface, having sky-blue fluorescence, and can be eluted with alcohol. F_2 moves down very slowly as a yellow-green, diffuse zone, which can be eluted with alcohol. Under the F_2 zone appears a reddish zone if some benzpyrene-5,8-quinone is present, and this can be washed out completely with the solvent. In the absence of F_1, X_2 appears as a sharp, narrow, pure blue zone at the top of the column, and X_1 is similar except that it extends for a few cm. This appearance does not change even on extended development. After cutting the X zones only small amounts of X_1 can be eluted with alcohol, and no X_2. Fluorescence spectrograms of the pure adsorbates show for the X-derivatives the same bands in the blue as the corresponding solutions (C. 2. Fluorescence Spectra, page 104), but in the case of F_1 and F_2 they are not identical, the following differences being found: for F_1 the adsorbate shows a broad, diffuse band from blue to green with no characteristic maxima, and for F_2 a shorter diffuse band from blue-green to yellow, whereas the corresponding solutions of F_1 and F_2 show two distinct maxima (see Fig. 3).

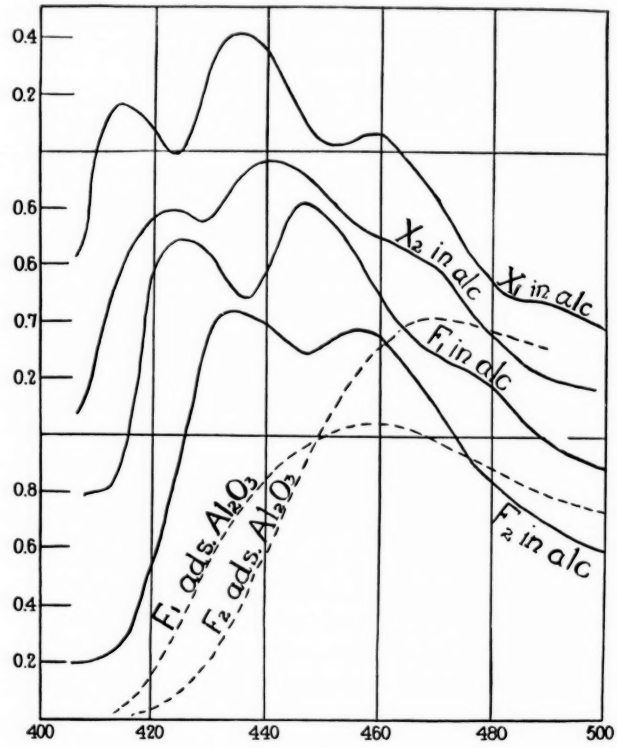


FIG. 3.—Fluorescence spectrograms of alcoholic solutions of X_1, X_2, F_1, and F_2. On the ordinates are plotted the densities D, at intervals of 0.2, of the spectrograms between 400 and 500 mμ recorded with a glass spectrograph on Kodak P. 800 plates. The densities of the various spectra cannot be compared except for the positions of the maxima, owing to the differences in fluorescence intensities, exposure, and development.

Adsorption on alumina from petroleum ether.—Benzpyrene and all derivatives are strongly adsorbed at the top of the column. F_2 does not move down.

Adsorption on alumina from amyl alcohol.—Only the X-derivatives are strongly adsorbed.

Adsorption on silica from aromatic hydrocarbons.—When all four derivatives are present only the X-derivatives are strongly adsorbed, forming a diffuse zone at the top of the column, with blue fluorescence. They can be partially but not completely eluted with methyl or ethyl alcohol. F_2 is not adsorbed, but F_1 forms a blue-fluorescent, diffuse zone on the column, which moves slowly down and can be washed out completely with the solvent. If any quinone is present it forms a red-fluorescent zone, which moves down

more slowly than F_1. The fluorescence spectra of the adsorbates of X_1, X_2, and F_1 are the same as those of the respective solutions.

Adsorption on silica from petroleum ether.—X_1, X_2, and F_1 are strongly adsorbed to the top of the column as diffuse zones. F_2 moves down slowly as a blue-fluorescent, diffuse zone with the same spectrum as its solution.

Adsorption on silica from amyl alcohol.—Only X_2 is adsorbed, forming a whitish-blue, diffuse zone at the top of the column, which can be washed out almost completely with alcohol.

Unchanged molecular benzpyrene is adsorbed to alumina only from petroleum ether; in all other cases it appears in the filtrate.

(*b*) *Scheme of Analysis.*—

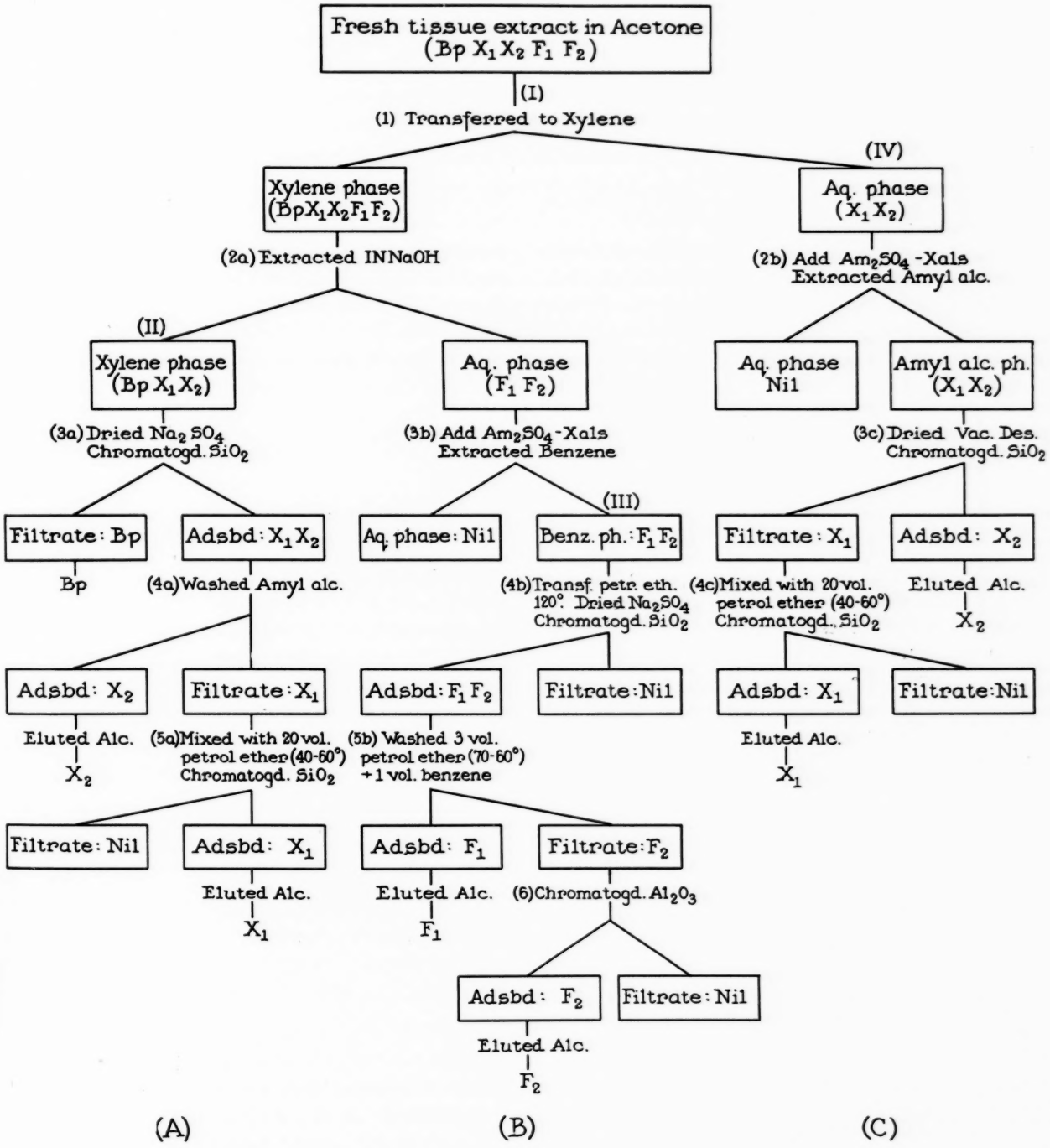


CHART I

(*c*) *Discussion of the Scheme.**—All tissue extracts usually fluoresce blue. However, if no trace even of blurred bands appears in the fluorescence spectrum in the case of a particular extract, it contains so small an amount of benzpyrene derivatives that it is not worth while to continue the operation. If there are bands in the blue portion of the spectrum the separation (1) in the Scheme must be carried out. If X-derivatives are present the xylene phase, and particularly the water phase, will fluoresce blue with banded spectra, and if F-derivatives are present as well the whole Scheme must be carried through. If no distinct F-fluorescence can be seen in the tissues the amount of F-derivatives usually is so small that the central column (B) of the Scheme may be omitted. On the other hand, in the absence of X-derivatives the aqueous phase of operation (1) contains no fluorescent derivatives with banded spectra, and only the central column (B) and operation (3a) for the separation of unchanged benzpyrene need be carried through. Usually the X-derivatives present in the xylene phase of operation (1) can be separated according to column (A) before working up the water phase by column (C), because the amyl alcohol extracts must be dried in the vacuum desiccator. In order to avoid undue losses the silica column of operation (3a) may then be used for operation (3c) as well.

If extracts of tissues under unknown conditions are being studied a short qualitative preliminary test with 0.25 to 0.5 cc. of the xylene extract passed through a double column having 2 cm. of silica above 2 cm. of alumina and washed with benzene gives useful information. In the presence of benzpyrene, X- and F-derivatives, and the benzpyrene-5,8-quinone a blue zone at the top of the silica column indicates X_1 or X_2. A sky-blue zone at the top of the alumina that does not move down on washing with benzene indicates F_1, and a yellow-green zone moving down slowly through the alumina column indicates F_2. Under the F_2 zone the quinone appears as a yellow-red, diffuse zone moving down more quickly than the F_2. Unchanged benzpyrene appears as a violet-fluorescent filtrate. Since the quinone is a secondary oxidation product of the derivatives and has nothing to do with the metabolism of benzpyrene, its separation was not included in the Scheme.

The addition of crystals of ammonium sulphate in operation (3b) to the yellow-fluorescent sodium hydroxide solution of the F-derivatives from operation (2a) liberates the very weak F-acids for extraction with benzene. The addition of some crystals in operation (2b) has merely a salting-out effect, helping to complete the extractions of the X-group with amyl alcohol. If these derivatives are moist from operation (1) and they are left in the vacuum desiccator with moist amyl alcohol for some days (3a) a slight transformation from X to F will often occur. The F-derivatives then appear in the blue-fluorescent filtrate of operation (4c). They may be separated, if necessary, from this filtrate by chromatography on alumina, where they appear as a yellow-fluorescent zone with no bands in the spectrum, moving slowly down. During the fluorescence spectrographic examination of the various stages it is to be borne in mind that the adsorbates of benzpyrene and of the X-derivatives on alumina and silica and of their solutions show bands in the blue-violet, whereas those of the F-group show no distinct bands as adsorbates on alumina but only as solutions and as adsorbates on silica. The pure typical fluorescence spectra of the various compounds appear only after complete separation. The benzpyrene-5,8-quinone shows a distinct red-yellow fluorescence only as adsorbate, and its presence can be detected even in small amounts by a diffuse increase of the density at the red end of the spectrograms. It must not be confused with sharp bands in the red that sometimes appear and are due to the presence of bile and urinary pigments or to chlorophyll derived from green food.

* Numbers within parentheses in this section refer to Chart I, not to bibliographical references.

C. Properties of X_1, X_2, F_1, and F_2

1. Solubilities

Water: X_1, X_2; alcohols: X_1, X_2, F_1, F_2; acetone: X_1, X_2, F_1, F_2; ether: F_1, F_2; aromatic hydrocarbons: X_1, F_1, F_2; petroleum ether: F_1, F_2.

2. Fluorescence spectra

The fluorescence spectra of blue-fluorescent solutions of the compounds differ according to whether glass or quartz spectrographs are used. The intensity of the violet and near-ultraviolet portions of the spectra is reduced with a glass as compared to a quartz instrument, owing to the absorption of short wave lengths by the heavy flint glass prism. Hence the intensity and maxima of the bands as recorded in Fig. 3 with the photoelectric device from the spectrograms exposed in the glass spectrograph are not identical with those published by other authors (1).

The fluorescence spectra of the derivatives are all of the same type, and not very specific. They differ mainly by the position of the two principal bands in the blue and violet. Owing to slight displacements of the bands in various solvents, only the fluorescence spectra in absolute ethanol are recorded in Fig. 3.

X_1 solution: color, pure blue. Two diffuse maxima at about 421 mμ and 440 mμ and an inflection (which appears in the spectrogram as a contrast band) at about 470 mμ. *As adsorbate to alumina or silica* it has the same color and spectrum.

X_2 solution: color, pure blue. Two diffuse maxima

at about 424 mμ and 445 mμ and an inflection at about 470 mμ. *As adsorbate,* same color and spectrum.[3]

F_1 solution: color, pure blue. Two diffuse maxima at about 420 mμ and 445 mμ. The first band rises more sharply from the violet than the corresponding X-bands. *As adsorbate to silica,* same color and spectrum. *To alumina,* color, sky-blue, one broad band from violet to yellow-green.

F_2 solution: color, pure blue. Two diffuse bands with maxima at about 435 mμ and 460 mμ, the first band rising sharply from the violet. *As adsorbate on alumina,* color, yellow-green, one broad band from blue to yellow without a banded structure.

3. ABSORPTION SPECTRA

The long ultraviolet portions of the absorption spectra of the metabolites are graphed together in Fig. 4 by plotting over a wave length abscissa log log I_0/I; *i.e.,* the logarithm of the "optical density" of the various solutions. This form of representation has the advantage that graphs for any individual absorbing substance always have the same shape, independent of the concentration or thickness of the absorbing solution (21A). Although the chemical constitutions and the molecular extinction coefficients, ε, of our derivatives are not known, log ε plotted over a wave length abscissa would give the same type of graph. For ease of comparison the curves are arbitrarily spaced in Fig. 4 together with those for 3,4-benzpyrene at the top and 8-hydroxy-benzpyrene at the bottom (the latter taken from the published spectrum [1] of the purified phenol). The solvent in all cases was ethanol.

Benzpyrene: The curve is in agreement with that published in the literature (15, 18), having three main isolated maxima at 348 mμ and 366 mμ and a doublet at 378 mμ and 385 mμ. A sharp, much weaker maximum at 393 mμ appears near the last strong band, and a sharp maximum in the violet at 404 mμ that is conspicuously detached from the main group of bands.

X_1: The absorption curve can be described as a broad band between 340 mμ and 400 mμ from which five secondary bands protrude, having maxima at 353, 363, 369, 380, and 388 mμ. In the violet is a single detached band with its maximum at 410 mμ. In the spectrograms the maximum at 380 mμ is most conspicuous as a narrow band.

X_2: This has a broad band from 340 to about 405 mμ from which four secondary bands with maxima at 344, 360, 378, and 390 mμ protrude. They have the appearance of being produced by coalescence of the five ultraviolet X_1 bands. The detached maximum in the violet at 412 mμ is less sharp than the corresponding X_1 maximum. In the spectrogram the bands appear less sharp than in the X_1 spectrum.

F_1: Three main isolated bands are present in the ultraviolet with maxima at 362, 380, and 395 mμ. There is a remarkably sharp detached maximum in the violet at 418 mμ. The spectrum is of the benzpyrene type, but the fine structure is missing.

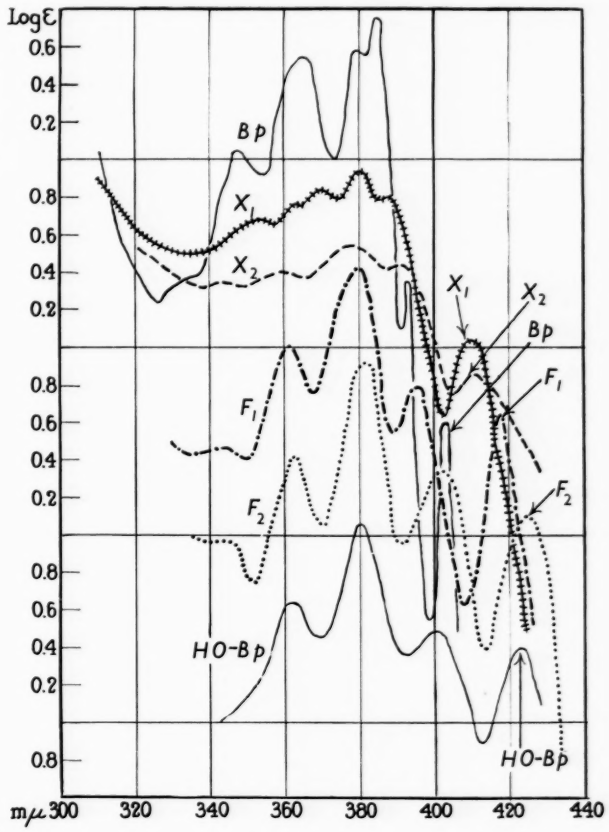


FIG. 4.—Absorption spectra (log log $I_0/I = \log \epsilon$) of benzpyrene (top), X_1, X_2, F_1, F_2, and pure 8-hydroxy-benzpyrene (bottom, from the literature [1]) dissolved in arbitrary concentrations in ethyl alcohol, between 300 and 440 mμ. The wave lengths of the last violet bands of the absorption spectra (marked by arrow) coincide with those of the first violet bands of the fluorescence spectra (Fig. 3).

F_2: The spectrum is very similar to that of F_1 and the first two ultraviolet bands have almost the same maxima at 363 and 382 mμ, but the third ultraviolet band and the detached band in the violet have maxima at 402 and 425 mμ, with a wave length difference of 7 to 8 mμ from the corresponding bands of F_1. In mixtures of F_1 and F_2 these last two bands, particularly the violet band, appear as doublets.

8-hydroxy-benzpyrene: The absorption spectrum taken from (1) is indistinguishable from that of F_2.

[3] The fluorescence spectrum of tissues due to "BPX" or "tissue-BP-blue" corresponds to that of X_2.

4. THE CHEMICAL PROPERTIES OF THE METABOLITES

While the X-derivatives do not show any pronounced acid properties in water, since their fluorescence is not affected by acids and alkalis, the F-derivatives are weak acids. They can easily be transferred with change of fluorescence from organic solvents to aqueous alkali and liberated again with acids. F_2 in particular acts as a phenolic fluorescence indicator, with a change of fluorescence color from blue to yellow between pH 9 and 10. Therefore ammonia does not dissolve it as a salt, and it can be liberated from its sodium or potassium salt solutions by the addition of saturated ammonium chloride or sulphate. This latter property was used in the Scheme for the separation of the metabolites.

The X-derivatives are sometimes transformed into F-derivatives in the living animal, and after death this transformation always occurs in X-bearing tissues in the absence of formol. After purification it was possible only in the case of X_1 to effect a transformation to an F-derivative (F_1) at room temperature; this could be carried out with dilute hydrochloric acid. Pure F_1 is formed in dilute alcoholic hydrochloric acid from X_1. In aqueous solution the transformation is slow with 0.5 *N* HCl at room temperature, but complete within a few minutes at 100° C. However, at the elevated temperature some 5,8-benzpyrene-quinone also appears in the chromatogram of the benzene extract.[4] If X_1 is heated to 100° C. with 0.5 *N* H_2SO_4 for several hours in the presence of air, only F_1 and the quinone are formed, but in vacuo F_2 is produced as well, with very little quinone.

Purified X_2 is stable in dilute alcoholic hydrochloric acid at room temperature, but if X_2 adsorbed on alumina is heated in an evacuated tube to temperatures above 150° C. the fluorescence changes quickly from blue to yellow-green. The eluate in alcohol shows the typical bands and chemical properties of F_2.

Quantitative estimation of the various compounds.— Because neither of the four derivatives of benzpyrene could be isolated in the pure crystalline form free from absorbent contaminants, it was impossible to determine their molecular extinction spectra by absorption spectrophotography, which would have enabled an exact quantitative analysis to be made. However, the following considerations provide a means for approximately estimating the amounts of X_1, F_1, and F_2 produced in the course of the metabolic conversion of benzpyrene.

From the graphs in Fig. 4 it is evident that the spectra of F_1 and F_2 are of a similar type to that of benzpyrene. As will be discussed in the following communication, this similarity shows that the benzpyrene derivatives F_1 and F_2 contain the aromatic benzpyrene ring system unchanged. Much spectrophotometric evidence justifies the conclusion that in such a case the molecular extinction spectra of aromatic compounds and of their simple alicyclic derivatives are quantitatively of the same order (15). This means that the same number of molecules of an aromatic compound and of its simple derivatives absorb light at the wave length of maximal absorption approximately to the same extent (15, 18). Hence it is possible to estimate the amount of the two F-derivatives from the extinction of the maxima of their strongest band at 380 mμ. It is known (15) that at 385 mμ the extinction of 1 mol of benzpyrene per liter in a cell of 1 cm. length is 30,000. Therefore 0.01 mgm. benzpyrene per cc. in a cell of 1 cm. length corresponds to an extinction of $\frac{30{,}000}{252 \cdot 100} = 1.2$. Because the benzpyrene maximum at 385 mμ is very sharp, whereas the F maxima at 380 mμ are more diffuse, the molecular extinction of the F-derivatives is probably slightly smaller than that of benzpyrene. Therefore we adopt the extinction 1.0 in a cell of 1 cm. length as corresponding to the amount of F-derivatives obtained from 0.01 mgm. benzpyrene per cc. An estimation of the relative amounts of F_1 and F_2 in a mixture necessitates a complete separation of both.

The amount of X_1 cannot be related directly with that of benzpyrene by this principle, because the type of its absorption spectrum is quite different from the benzpyrene type. However, by making use of the easy transformation of X_1 into F_1 the ratio of the maximal extinction of X_1 to that of F_1 can be established. If this transformation is carried out in 2.5 cc. of a methyl alcoholic solution of X_1 of suitable concentration, purified by chromatography, by adding 0.05 cc. of concentrated hydrochloric acid and leaving the mixture for one hour at room temperature, X_1 is quantitatively transformed to F_1. The average ratio E_{X_1}/E_{F_1} of the extinction maxima as determined by a number of such experiments is 1.2/1.4. Hence we adopt the extinction maximum of X_1 of 0.85 at 380 mμ in a cell of 1 cm. length as corresponding to the amount of X_1 derived from 0.01 mgm. benzpyrene per cc.

At the present stage it is not possible to estimate the

[4] This reaction provides a method for the preparation of F_1: to a crude acetone solution of the X-derivatives (*e.g.*, from the small intestine) or to an alcohol solution of purified X_1 are added a few cc. of water. The acetone or alcohol is then removed under reduced pressure, an equivalent volume of 1.0 *N* HCl is added, the mixture kept at 100° C. for 10 minutes, cooled, extracted with benzene, and the benzene phase dried with anhydrous sodium sulphate. Then the benzene solution is allowed to percolate through a chromatographic tube with a column of 3 cm. of silica above a column of 4 cm. of alumina, and developed with benzene until the red-fluorescent quinone has moved well below the sky-blue F_1, which is adsorbed at the top of the alumina and is eluted with methyl alcohol. Any unchanged X-derivatives are retained on the silica column.

amount of X_2 by a similar method because it cannot be separated from X_1 except by adsorption to silica from amyl alcohol, and a solvent for its complete elution has not yet been found. However, since the absorption spectra of X_1 and X_2 are of similar type there will be no great error if both are estimated in mixtures.

The estimation of the various metabolites of benzpyrene by these indirect methods was developed because we wished to follow the course of the metabolism of the hydrocarbons under various experimental conditions. The detailed results will be given at a future time. Since any attempt to purify the tissue extracts by chromatography and other operations invariably involves serious losses, the estimations must be carried out with that solution which contains the derivative in question at its maximum amount, and in such a degree of purity that its specific absorption spectrum can be clearly recorded in the spectrogram. This for the F-derivatives is the crude acetone extract from the feces and minced large intestine that has been freed from adherent violet-fluorescent fat as completely as possible. For X_1, it is either the acetone extract of the minced tissue or, after hydrolysis of the tissue with methyl alcoholic potash, the filtrate obtained by passing the amyl alcohol extract through silica and subsequently washing. The silica column retains X_2.

Both these F and X preparations invariably contain, apart from the metabolite in question, large amounts of absorbing impurities, and the color of the F extracts is sometimes distinctly yellowish. The problem of a spectrographic analysis of carcinogenic hydrocarbons and metabolites under such conditions has been studied in a number of communications by Jones (15-17). Jones compared the complete log ϵ spectrum of the impure solution with a "master curve" of the log ϵ spectrum of the pure hydrocarbon or its derivative. Owing to the impurities the experimental log ϵ spectrum is always higher than that of a pure solution containing the same amount of the compound. By drawing the master curve on transparent paper it is possible to adjust its ordinates so that it touches the experimental curve but never lies above it. From the extinction at the wave length where they coincide the maximum amount of the compound in question present in the impure preparation can be estimated. Although this procedure, which considers a number of points of the curve, is certainly better than ours, we established by a number of experiments with contaminated solutions of benzpyrene that the simple comparison of the maximum extinction gives estimates of the right order.

SUMMARY

After reviewing existing knowledge of the metabolism of benzpyrene, methods are described serving for the separation, purification, identification, and estimation of four different products of the metabolic conversion. These are symbolized by X_1, X_2, F_1, and F_2, and some of their properties are described.

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The Biochemistry of Benzpyrene

II. The Course of Its Metabolism and the Chemical Nature of the Metabolites*

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The Course of the Metabolic Conversion

Some of the experiments that led to the conclusions summarized in the Introduction to our earlier paper (32) were repeated with the improved methods, and the older fluorescence spectrograms were also re-examined. The general results with respect to the fluorescent appearance of "BPX," "tissue-BP-blue," and "BPF" at the various sites were confirmed. However, since it is now known (32) that these fluorescences are due to complex mixtures of 4 different derivatives, X_1, X_2, F_1, and F_2, slight differences in the spectra to which no great importance was attributed previously can now be explained by the preponderance of one or other of these derivatives. Their relative amounts, now to be given, are in many cases derived from estimates of the intensity of the fluorescence spectra, except when numerical data are presented based on absorption spectrophotography.

1. Liver and gall bladder.—Although unextracted liver showed exclusively the X_2 fluorescence spectrum, its extract emitted the X_1 fluorescence. The presence of X_1 always reveals itself by a strongly blue-fluorescent xylene phase in operation 1 of the Scheme of Analysis described in the preceding publication (32). The minced liver tissue loses its fluorescence almost completely after one single extraction with acetone. This is an indication for the absence of much X_2, which is usually strongly held by the tissue. If in a preliminary experiment the xylene extract is filtered through a short column of alumina, X_2 forms a bright blue-fluorescent thin surface zone, whereas X_1 gives a diffuse blue zone extending in the case of the liver extract about 2 cm. into the column. The examination of these qualitative phenomena gives quite reliable evidence of the relative amounts of the two X-derivatives. The storage of liver tissue before its extraction yields F_2; see (32) Introduction: ¶ 8. Under the artificial conditions mentioned in Introduction: ¶ 11 of the same paper, the liver tissue contains much X_2.

2. Bile.—If the opened gall bladder is extracted with acetone the amount of X_2 in the clear extract is usually much larger than in the liver extract, whereas the unextracted sediment and the gall bladder wall contain almost exclusively X_2, which cannot be recovered except by alkaline hydrolysis (32). F_2 is found after storage of the gall bladder before extraction.

3. Small intestine.—After intravenous inoculation of a finely dispersed benzpyrene colloid the blue-fluorescent first acetone extract of the minced tissue contains much X_1 and little X_2. In successive acetone extracts the relative amount of X_2 increases more and more until the strongly fluorescent tissue is left, containing almost exclusively X_2. Part of this can be extracted with boiling amyl alcohol, and the rest by alkaline hydrolysis. Numerous experiments with the tissue of the small intestine established the following rule: the less the concentration of X-derivatives entering the small intestine with the bile, the less is the proportion of extractable X_1 in the mixture of the two X-derivatives. This concentration is controlled by the amount of benzpyrene transported to the liver by the blood stream. It is high only in the first 1 to 2 hours if a large amount of finely dispersed benzpyrene colloid is inoculated intravenously, but after that time the concentration of dissolved benzpyrene in the blood remains fairly constant for some hours and then drops slowly. The level is maintained owing to the storage of benzpyrene in the lung capillaries, which trap particles after inoculation of a coarse suspension, and in the adipose tissue. After intraperitoneal injection of 10 mgm. the benzpyrene content of the blood is, according to Berenblum and Schoental (4), stable at about 0.0005 mgm. per mouse for 5 days, although the weight of benzpyrene in the whole mouse drops to 2 mgm. After the deposits are exhausted the benzpyrene level of the blood falls, and X_2 is almost exclusively found in the small intestine. This happens

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† Dr. Mottram died on October 4, 1945.

earlier in larger animals, such as rats and rabbits, because it is not easy to inoculate into the blood stream amounts of colloid equivalent to those introduced into mice.

When the small intestine of a mouse is stored in a refrigerator before extraction, much X is transformed into F after 3 days; practically no X is left after 1 day at room temperature, or 3 hours at 37° C. (17).

A few quantitative attempts were made to determine the approximate yield of X_1 in the course of the metabolism. In one experiment 0.5 mgm. of benzpyrene was inoculated into the tail vein of a mouse as a finely dispersed colloid and the animal was killed after 100 minutes. No F-derivatives had reached the cecum during that period, but the liver, gall bladder, and entire small intestine fluoresced strongly blue; these were minced and extracted twice with acetone. The acetone extracts showed clearly the X_1 maximum at 380 mμ in their extinction spectra, from which it was established that the liver+gall bladder contained about 0.04 mgm., and the small intestine about 0.19 mgm., of X_1. Since 0.18 mgm. unchanged benzpyrene was found in the carcass the yield of X_1 from the metabolized portion of the benzpyrene was about $\frac{0.23 \cdot 100}{0.5-0.18} = 72$ per cent. In two other experiments the yields were 82 and 74 per cent. Taking into account that the wall of the small intestine after extraction with acetone was still strongly blue-fluorescent, due to X_2, and that some X-derivatives were seen in the kidneys, it can be concluded that the yield of X_1 accounts for most of the metabolized hydrocarbon.

In another experiment the entire small intestine was hydrolyzed with methyl alcoholic potassium hydroxide after an intravenous inoculation of 0.5 mgm. of benzpyrene as a finely dispersed colloid. The mouse was killed 90 minutes later, and the X_1 estimated in the amyl alcohol extract after its passage through silica in order to remove X_2. In this case 0.07 mgm. of X_1 and about 0.22 mgm. of unchanged benzpyrene were found. From these data it follows that the yield of purified X_1 from the small intestine was about $\frac{0.7 \cdot 100}{0.5-0.22} = 25$ per cent. The yield was smaller than in the first case since the liver+gall bladder and kidneys were not analyzed, and because of losses in the course of purification by this less efficient method.

4. Large intestine and feces.—The feces in the large intestine and those collected are easily extracted with acetone after mincing; the extraction is not quite complete, since some blue-green-fluorescent particles remain. The extract contains F-derivatives almost exclusively, apart from traces of X_1 and unchanged benzpyrene. The presence of two different derivatives in the extracts is indicated by a blurred fluorescence spectrum that shows the F_2 bands indistinctly and diffusely extended towards the ultraviolet. In the absorption spectra of the crude acetone extracts the 2 characteristic maxima in the violet at about 418 mμ and 425 mμ are seen. In the presence of much F_1 a bluish-white top zone appears in the fluorescence chromatogram on alumina, from which the yellow-green diffuse zone of F_2 separates slowly on development with benzene.

The relative distribution of the two F-derivatives in the acetone extract of feces changes according to the following rules if the material is collected in successive portions: The fluorescent feces that appear first after an intravenous inoculation contain much F_1 and little F_2. In later collections the relative amount of F_2 increases more and more compared with that of F_1. The predominance of F_1 in the first fractions of feces is less conspicuous after an intraperitoneal inoculation of benzpyrene. The separation of the two F-derivatives by Chart I (32) is not complete, but if the feces are collected from the second day after intraperitoneal inoculation F_2 is produced almost exclusively.

If, after intravenous inoculation, the large intestine is subjected to alcoholic hydrolysis almost pure F_1 can be recovered, because F_2 is decomposed by strong alkali to a greater extent than F_1. However, this mode of preparation involves greater losses than the milder extraction of the F-derivatives with acetone.

Attempts to determine the approximate yield of the F-derivatives were made in some cases. In these experiments the feces were collected in acetone in a number of successive fractions after the intravenous administration of benzpyrene to mice. When the fluorescence of the acetone extracts was weak the mouse was killed. The amount of F-derivatives was estimated by spectrophotometry at 380 mμ either in the original crude acetone extracts or after their transfer to petroleum ether (boiling above 120° C.), according to operation 4b of the Scheme of Analysis (32). Treatment of the carcass to determine the amount of unchanged benzpyrene present showed that after 1 or 2 days the amount was very small, and therefore in calculating the yield of F-derivatives the entire inoculated amount was used. On the other hand, after intraperitoneal inoculation of a 0.5 per cent solution of benzpyrene in sesame oil the amount of unchanged hydrocarbon in the peritoneum must be taken into account, as well as those F-derivatives that can be extracted from the large intestine.

Berenblum and Schoental have shown that the disappearance of benzpyrene conforms to a monomolecular process, and from their published diagram the value 0.15 was taken as the constant, k, in the equation $\log c_o/c_t = kt$ for a monomolecular reaction where c_o is the intraperitoneally inoculated benzpyrene, and c_t

the hydrocarbon left in the carcass after t days. Since our estimates of the amount of residual unchanged benzpyrene fit well into the published graph, we have used in the following table for calculation of the yield of metabolites in Experiments 4 to 6 the amount of hydrocarbon that had disappeared as calculated by this formula. As a small difference between two large quantities, it is less liable to errors than a direct determination.

Table 1: Experimental Determination of the Amount of Benzpyrene Excreted as F-Metabolites

The times shown in each of the experiments, 1 to 6, are from left to right the consecutive periods during which the feces were collected. Weights in mgm., ° = hours, ′ = minutes.

1. *0.5 mgm. benzpyrene intravenously inoculated into 1 mouse*

	24°	24°
Metabolized benzpyrene	0.19	0.0

Total yield: 38% in 1st acetone extract.

2. *0.5 mgm. benzpyrene intravenously inoculated into 1 mouse*

	5°35′	5°15′	8°40′	12°	
Metabolized benzpyrene	0.115	0.095	0.095	0.0	Total 0.305

Total yield: 61% in 1st acetone extract.

3. *1.2 mgm. benzpyrene intravenously inoculated into 3 mice*

	18°	12°	
Metabolized benzpyrene	0.34	0.04	Total 0.38

Total yield: 31% in petroleum ether.

4. *5 mgm. benzpyrene intraperitoneally inoculated into 1 mouse*

	12°	12°	large intestine
Metabolized benzpyrene	0.12	0.17	0.08

Benzpyrene disappeared (calculated): 1.47 mgm. Total yield: 25% in 1st acetone extract.

5. *5 mgm. benzpyrene intraperitoneally inoculated into 1 mouse*

	15°30′	8°30′	18°30′	22°30′	22°30′	large intestine	
Metabolized benzpyrene	0.136	0.135	0.213	0.186	0.101	0.057	Total 0.83

Benzpyrene disappeared (calculated): 3.75 mgm. Total yield: 22% in 1st acetone extract.

6. *15 mgm. benzpyrene intraperitoneally inoculated into 3 mice*

	13°	11°	11°35′	12°25′	11°30′	12°20′	12°40′	9°	13°	13°	large intestine	
Metabolized benzpyrene	0.096	0.130	0.192	0.152	0.159	0.136	0.240	0.115	0.128	0.232	0.072	Total 1.652

Benzpyrene disappeared (calculated): 12.33 mgm. Total yield: 13.5% in petroleum ether.

On comparing the amounts of the excreted F-derivatives in the 6 experiments it can be seen that after intravenous inoculation of benzpyrene (Experiments 1 to 3) almost the entire amount of benzpyrene is metabolized during the first 24 hours, but after intraperitoneal inoculation (Experiments 4 to 6) the excretion is rather evenly distributed over all fractions, although the disappearance of the benzpyrene—after a reaction of the first order—slows down considerably with increasing periods of inoculation. This puzzling discrepancy is especially obvious in Experiment 6, as can be seen from Fig. 1, the widths of the columns corresponding to the periods during which the various portions of feces were collected, the total heights of the columns to the respective (calculated) amounts in mgm. of the disappearing benzpyrene, and the heights of the black columns to the amounts in mgm. of metabolized benzpyrene estimated as F-metabolites. The last short shaded column shows the F-contents of the large intestine after killing the mice.

It is to be noted that the estimated yields after chromatographic purification (Experiments 3 and 6) are smaller than the estimates from the crude first acetone extracts (Experiments 1, 2, 4, and 5).

5. Kidney.—The blue fluorescence of the kidney cortex appears about 30 minutes after intravenous inoculation of the colloid. The acetone extract, which removes the blue fluorescence almost completely, contains both X-derivatives. When chromatographed on alumina from xylene the blue diffuse top zone containing X_1 does not extend so far down the column as the corresponding X_1 zone from the liver. The absorption spectrum of kidney-X_1 shows the same bands as X_1 from the small intestine and liver. After storage of kidney tissue before its extraction some F_2 is produced.

6. Urine.—Owing to difficulties in collecting sufficient amounts of uncontaminated mouse urine and to the presence of strongly fluorescent urinary pigments no definite statements can be made, although there are indications that the X-derivatives are present in small amounts. However, an experiment with a rabbit from which a filled bladder was dissected showed the following phenomena. The fresh urine drawn from the bladder did not show any specific fluorescent benzpyrene bands, but after storage for 3 days at room

temperature the pure F_1 fluorescence spectrum was found, in spite of the fact that the kidney cortex of this rabbit developed on storage a pure F_2 fluorescence. This is an indication that X_1 derivatives had previously been present in the urine. However, these experiments

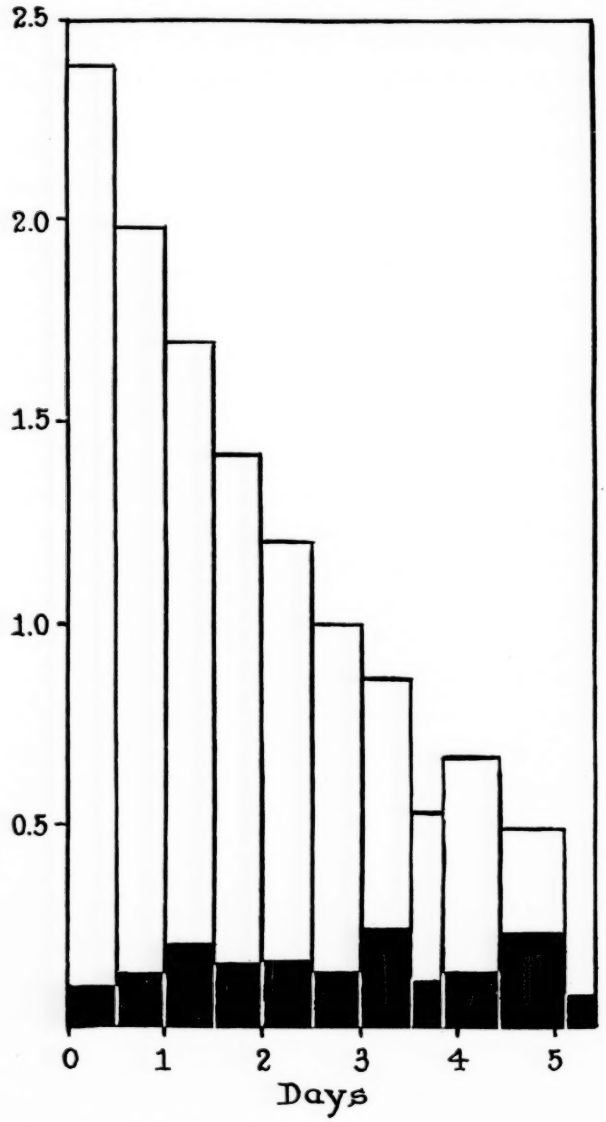


FIG. 1.—Rate of excretion of benzpyrene after intraperitoneal inoculation of 15 mgm. benzpyrene into 3 mice (Table I, Experiment 6) during 5 days. The total heights of the columns correspond to the (calculated) amounts (in mgm.) of benzpyrene that disappeared in successive periods; the heights of the black columns to the amounts (in mgm.) of benzpyrene recovered as F-derivatives.

with rabbits are not quite conclusive for the mouse, because according to Berenblum and Schoental (5) the metabolism of benzpyrene in the rabbit is not the same as in the mouse.

7. Lung.—After intravenous inoculation of a finely dispersed benzpyrene colloid into a mouse, the lung usually contains extremely small amounts of the X-derivatives. On the other hand, much more of the metabolites appears after inoculation of coarse suspensions, the large particles of which are retained for several hours in the lung capillaries. The absorption spectrum of the extract shows chiefly the X_1 bands. After intravenous inoculation of a coarse suspension into rabbits the following phenomena were observed in some cases: On inspection in ultraviolet light the fresh lung fluoresced violet from unchanged benzpyrene, but more or less extended areas fluoresced brilliantly blue-green. These portions showed the typical reactions of F_2: when dissolved in strong alkali they fluoresced yellow-green, which turned blue-green with dilute acid. On extraction of the lung with acetone, much X_1 as well as F_2 could be recovered. In one case, when the inoculated rabbit died after 1 hour for unknown reasons, the entire outer surface of the lung showed the blue-green fluorescence. This tissue was extracted with cold acetone and then with boiling ethanol. Apart from much unchanged trapped benzpyrene, X_1 and F_2 were identified by fluorescence spectrography. This alcohol extract was inspected 4 years later after storage in a dark place. After this time no more F_2 could be found, but only unchanged benzpyrene, X_1, and large amounts of benzpyrene-5,8-quinone. This indicates that X_1 is stable over long periods under the conditions of the experiment, whereas the F-derivative was oxidized to quinone. The presence of X after so long a time indicates that X does not even slowly change to F in alcoholic solution.

8. Mammary glands.—Mice were inoculated with coarse benzpyrene suspensions 4 days after littering. The suckling young were killed 2 to 4 hours later, and the stomachs opened under water and extracted with ether, which removed some molecular unchanged benzpyrene; the aqueous phase was precipitated with ammonium sulphate and extracted with amyl alcohol, which showed the X_1 fluorescence spectrum more or less distinctly. In some cases there appeared a band at about 418 mμ, indicating the presence of F_1, the production of which is probably due to the acidity of the stomach contents.

9. Subcutaneous tissue.—In one experiment a mouse was inoculated subcutaneously in one flank with 0.2 cc. of mouse fat [1] containing 0.1 per cent benzpyrene, and in the other flank with 0.2 cc. of sesame oil containing 0.1 per cent benzpyrene. The mouse was killed 3 days later, the inoculated tissues were dissected out, and the acetone extracts tested in the usual way. Apart from large amounts of unchanged benzpyrene in both flanks, X_2 could be recovered from the tissue inoculated with mouse fat, but no benzpyrene derivatives, or only very little, from the tissue inoculated with sesame oil. The

[1] A sample was kindly supplied by Dr. F. Dickens, Newcastle-upon-Tyne.

histological examination of portions of the tissues showed differences with respect to their reactivity, the detailed study of which is in progress.

10. Skin.—The fluorescence of the skin of numerous mice has been studied after painting with solutions of benzpyrene in acetone and in benzene. The experiments were varied over a wide range and led to the results previously reported, that BPX appeared in the cells of the malpighian layer and could be detected there qualitatively, even after a period of 3 weeks following 1 single painting. We know now that this fluorescence spectrum of BPX corresponds to that of X_2. After hydrolysis with methyl alcoholic potassium hydroxide mainly X_2 could be recovered from the extracts from skins that were studied 24 hours after painting.

A number of quantitative experiments were made recently in order to establish the metabolism of painted benzpyrene under various conditions. In one case a mouse was painted on the clipped skin of both flanks, each with 0.03 cc. of 0.3 per cent benzpyrene in acetone; the total amount of hydrocarbon administered was 0.18 mgm. The mouse was placed in a glass box in order to estimate the loss of solid benzpyrene from the coat within the first 4 hours. It amounted to less than 0.0004 mgm. The benzpyrene excreted in the feces in the F-form was 0.06 mgm. in the first 24 hours and an additional 0.021 mgm. in the next 24 hours. The mouse was then killed and the skin, which still fluoresced superficially weakly violet, was extracted with benzene. The loosely fixed benzpyrene so removed amounted to about 0.003 mgm. The fluorescence of the dissected organs did not show any detectable benzpyrene derivatives. They were added to the carcass and hydrolyzed with alcoholic potassium hydroxide, which yielded about 0.003 mgm. of unchanged benzpyrene. Some X_2 was recorded in the extracts from the hydrolyzed skins, but could not be estimated quantitatively. Taking into account the lack of precision in the amount of benzpyrene applied, and in the analyses, roughly 50 per cent of the absorbed benzpyrene was excreted as F-metabolites in the feces. This agrees well with the amount of F_2 estimated after intravenous and intraperitoneal inoculation.

Another set of experiments was carried out in order to establish the way in which benzpyrene disappears from, and X-derivatives appear in, the painted skin, by comparing the density of the maxima of their fluorescence spectra. One example may be given, showing at the same time the influence of pre-painting with croton oil, which has been shown (26) to sensitize the skin to tumor formation. In a number of provisional experiments, it had been established that after pre-painting the skin with croton oil or croton resin the hydrocarbon disappeared from the skin more quickly than without this pre-treatment.

Eight mice were painted 5 times with croton oil on alternate days on their right flanks. Two days after the last croton oil treatment they were painted on both flanks with 0.03 cc. of a 0.3 per cent solution of benzpyrene in acetone. These paintings were timed in such a way that, on killing the mice in quick succession, the skin had been in contact with the hydrocarbon for 0, 2, 4, 8, 16, 24, 32, and 48 hours. The 16 samples of skin were then detached and washed for 24 hours with frequently changed benzene (2 cc. of benzene at a time). The benzene wash containing the loosely fixed benzpyrene was pooled in 16 separate lots. The skin was then hydrolyzed, each sample with 1 cc. of saturated potassium hydroxide solution and 5 cc. of methyl alcohol, and left in the cold alkaline mixture for 24 hours. After addition of 5 cc. of saturated ammonium chloride solution the alcohol was evaporated at reduced pressure and the liquid was then extracted with 2 cc. of amyl alcohol. The amyl alcohol extracted the hydrocarbon fixed to the tissue and the X-metabolites formed during the contact. Then, after the fluorescence spectrograms of the extract had been recorded, the hydrocarbon was separated by passing the amyl alcohol extract through a column of silica, and the top zones were eluted with methyl alcohol. The fluorescence of the eluate was again spectrographed, and found to be free from benzpyrene. All spectrograms were exposed and developed in the same way and the photographic densities were measured photoelectrically at the wave lengths of greatest fluorescence intensity. Since in the amyl alcohol extract the bands of the fixed benzpyrene overshadowed completely the X_2 fluorescence the benzpyrene maximum at 427.5 mμ was chosen, and for the X_2-containing eluates the X_2 maximum at 445 mμ. The photographic densities are plotted in Fig. 2 with a time abscissa. I_R and II_R correspond to the amounts of fixed benzpyrene and X_2 respectively in the sensitized right flanks of the mice, and I_L and II_L in the unsensitized left flanks at 0, 2, 4, 8, 16, 24, 32, and 48 hours after a single painting with benzpyrene. Although the conditions of this experiment were standardized at all stages, smooth curves could not be expected because 8 different mice were used.

However, it is quite evident that: (a) in all cases the amounts of fixed benzpyrene and of X_2 increase slowly in the skin and disappear again after passing through a maximum; and (b) on the control side the maxima are higher and reached later than on the sensitized side. The loosely attached benzpyrene, excess of which was painted on the skins and could be washed off completely with benzene, was not plotted in the figure. Just as in the preliminary experiments, it disappeared much more quickly from the sensitized flanks than from the control flanks

of the mice. The quantity of fixed benzpyrene when at its maximum is not more than about 1/100th of the amount painted on the skin.

11. Blood and plasma.—In agreement with Berenblum and Schoental (4) it was established that the amount of benzpyrene in the whole blood volume of a mouse from 1 hour after an intraperitoneal inoculation was of the order of 0.0005 mgm. No metabolites could be found under normal conditions, but after the common bile duct had been cut (18) X_2 accumulated in the plasma and could be detected there even

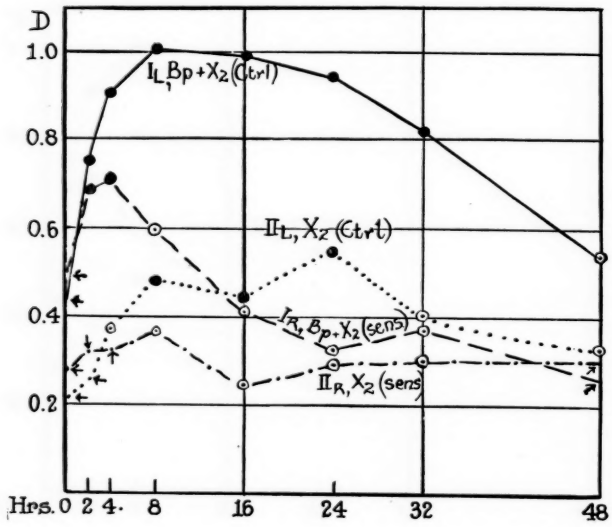


FIG. 2.—The fixation of painted benzpyrene and the production of X_2 in sensitized and control skins from the flanks of mice after 0, 2, 4, 8, 16, 24, 32, and 48 hrs., as measured by the photographic densities, D, of the fluorescence spectrograms. Visibility of bands: ● = strong; ⊙ = weak; → = no bands. I_R, benzpyrene + X_2 (sensitized); I_L, benzpyrene + X_2 (control). II_R, X_2 (sensitized); II_L, X_2 (control).

5 days after the operation. Under these abnormal conditions the X_2 fluorescence in the tissues of the liver, kidney, lung, and small intestine, where normally it disappears completely after 1 day, persisted for 5 days. Since it could also be seen as a new phenomenon even in the tissue of the stomach and large intestine, these long-persistent blue fluorescences are obviously due to a "staining effect" by the blue-fluorescent plasma.

DISCUSSION

The problems connected with the products of metabolic conversion of benzpyrene must be discussed from the chemical and physicochemical, from the biochemical, and from the biological standpoints.

A. The Chemical Nature of the Metabolites

Although it has not yet been possible to analyze the X- and F- benzyprene derivatives and to establish their constitution by purely chemical operations (particularly because they cannot be freed from other cell constituents without decomposition), their physicochemical properties narrow down considerably the chemical possibilities. The conclusions are based upon the following established facts: (a) The parent hydrocarbon is 3,4-benzpyrene; (b) X_1 appears *in vivo* only, as the first product of the metabolic conversion; (c) The end product is 8-hydroxy-3,4-benzpyrene; (d) The F-derivatives have pronounced phenolic or weakly acid properties and the X-derivatives have not; (e) The absorption spectra of the F-derivatives and of 8-hydroxy-benzpyrene are of the benzpyrene type, whereas those of X_1 and X_2 are of a different type but similar to each other; and (f) X_1 is transformed *in vitro* to F_1 by a mild chemical operation, whereas X_2 is stable under this treatment.

According to general experience the absorption spectra of aromatic hydrocarbons and those of their derivatives with unchanged aromatic ring system are of the same type (2, 9, 16, 21, 22, 23, 24, 25). Hence benzpyrene, F_1, F_2, and 8-hydroxy-benzpyrene must contain the same fully aromatic pentacyclic benzpyrene ring system. This was used (32) to devise an indirect method for an approximate quantitative estimation of F_1 and F_2 from the known extinction spectra of benzpyrene. On the other hand, it can be concluded from the fact that the absorption spectra of X_1 and X_2 are quite different from that of benzpyrene, that they do not contain the unchanged benzpyrene ring system. The metabolic conversion from benzpyrene into X_1 may involve any change of the molecule. No direct chemical conclusions can be drawn from transformations occurring *in vivo,* because they usually follow quite different mechanisms from those *in vitro.* However, the smooth and quantitative change from X_1 into F_1 in dilute alcoholic hydrochloric acid at room temperature is a very mild, purely chemical reaction, and it restores in F_1 the pentacyclic aromatic ring system of benzpyrene. Hence the difference between the aromatic structure of the mother substance, benzpyrene, and X_1 can be only very slight.

An exact model for such a sequence—change of an aromatic system *in vivo* and restoration in a mild chemical reaction—exists in the study of the metabolism of anthracene in rats and rabbits by Boyland and Levi (6). (See also Fig. 3.) When anthracene (I) is fed to rats and rabbits, it is excreted in the urine as 1,2-dihydroxy-1,2-dihydro-anthracene (II) of which the constitution, with one double bond less than anthracene, was established by purely chemical operations. It loses one molecule of water by the mild action of dilute hydrochloric acid and is transformed into 1-hydroxy-

anthracene (α-anthrol) (III), which again contains the complete aromatic system of anthracene.

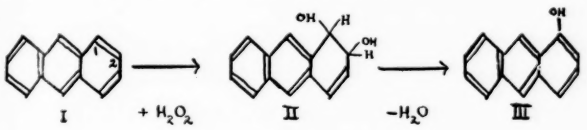


FIG. 3

Fieser (20) assumed that such an addition of hydrogen peroxide followed by loss of water, which he termed "perhydroxylation," also precedes the metabolic formation of 4′,8′-dihydroxy-1,2,5,6-dibenzanthracene after the administration of 1,2,5,6-dibenzanthracene to rats and rabbits. In this case the first metabolite after the addition of hydrogen peroxide would be expected to be an alcohol with two pairs of hydroxyl groups distributed in different rings and not in the same ring. Fieser even predicted that a phenolic metabolite of benzpyrene (Fig. 4), which was not known at that time, would not be expected to carry a hydroxyl group in position 5 (IV), for although this position is reactive to substitutions it is not amenable to additions. It is now known from the experiments of Berenblum and his associates (2, 3, 5) that the phenolic metabolite of benzpyrene discovered by Chalmers and Crowfoot (12) carries the hydroxyl group in the 8-position (VI); therefore a hypothetical direct precursor of 8-hydroxy-benzpyrene, which Berenblum anticipated in an (unpublished) address before the Biochemical Society in Oxford in 1942, ought to be a di-alcohol carrying the two hydroxyl groups at the positions 8 and 9 of the benzpyrene ring system (V).

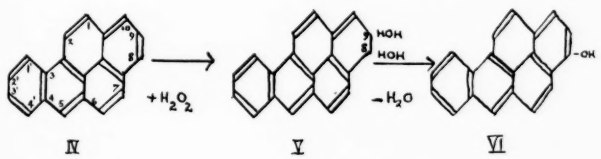


FIG. 4

According to our new experiments the precursor of 8-hydroxy-benzpyrene is no longer hypothetical, and it is necessary to consider how the properties of X_1, X_2, F_1, and F_2 fit into this image based on the metabolism of anthracene.

The absorption spectrum and the properties of F_2 indicate with little doubt that it is identical with the metabolite of benzpyrene that was first termed "BPF" (11, 17, 18, 27, 30, 31) and later "BPX" (2, 3, 5, 12, 24),[2] the chemical structure of which as 8-hydroxy-3,4-benzpyrene was based on crystallographic X-ray analysis (2, 12), absorption spectra, and its chemical relationship to 3,4-benzpyrene-5,8-quinone (2, 12). Therefore we have to consider only X_1, X_2, and F_1 as precursors. X_1 cannot be the di-alcohol 8,9-dihydroxy-8,9-dihydro-benzpyrene (V in Fig. 4), although its nonphenolic nature would be consistent with such a structure, because the smooth reaction with dilute hydrochloric acid yields F_1 and not the phenol 8-hydroxy-benzpyrene. Furthermore, the strong adsorption of X_1 to alumina would not be consistent with a di-alcohol.

F_1 must be very closely related to 8-hydroxy-benzpyrene because the absorption spectra of the two compounds are almost identical but for slight displacements of the violet bands (32, Fig. 4). An indication of the chemical nature of F_1 can be taken from the evidence given by Boyland and Levi (7) that, in addition to the metabolism of anthracene to 1,2-dihydroxy-1,2-dihydroanthracene, 1,2-dihydroxy-1,2-dihydro-anthracene-1-glucuronic acid is produced as well. This conjugate is easily transformed into 1-anthryl-glucuronic acid with dilute hydrochloric acid, and this in turn yields α-anthrol on prolonged boiling with 0.2 *N* sulphuric acid. Based upon this model reaction it is very likely that in F_1 8-hydroxy-benzpyrene is conjugated with a radical, R_1, derived from the cell, to form 8-OR_1-benzpyrene. The nature of R_1 has not yet been established, but the stronger adsorption of F_1 to alumina than to silica and its solubility in alkali indicate that R_1 has acid properties. The substitution of the phenolic H by R_1 would account for the close relationship between the absorption spectra of F_1 and 8-hydroxy-benzpyrene and, probably owing to the chemical nature of F_1, for its stronger adsorption to alumina than that of 8-hydroxy-benzpyrene. Furthermore, F_1 can be transformed into 8-hydroxy-benzpyrene by prolonged heating with sulphuric acid. Correspondingly, X_1, from which F_1 can be prepared by the mild action of dilute hydrochloric acid, must have the constitution 8(OR_1)-9(OH)-8,9-dihydro-benzpyrene. This formula accounts for the new absorption spectrum of X_1 (32, Fig. 4), because the 8,9 double bond of the aromatic benzpyrene ring system is hydrogenated, and the presence of the R_1 group is responsible for the strong adsorption of X_1 to alumina. For corroboration of this suggested structure of X_1 it would be helpful to synthesize a compound containing the residual aromatic 4-ring nucleus, which remains intact, in order to compare its absorption spectrum with that of X_1.

No information is yet available on the chemical constitution of X_2 except its close association with X_1, which can be inferred from the similarity of their absorption spectra. The long persistence of X_2 in the living animal in the skin, subcutaneous tissue, and plasma, under artificial conditions (18), and its much stronger adsorption to the columns in the

[2] In order to avoid any further confusion between these two terms they will no longer be used in this series of communications.

fluorescence chromatograms make it likely that X_2 is a product of the combination of X_1 with cell constituents, which may be symbolized by R_2. The simplest assumption would be that the 9(OH)-group of X_1 is linked with R_2 in X_2 to form 8(OR_1)-9(OR_2)-8,9-dihydro-benzpyrene. Such a compound, having its free hydrogens substituted, would be expected to be more stable towards chemical agents. This is in agreement with the fact that the aromatic benzpyrene nucleus is not restored by the mild treatment with dilute hydrochloric acid, but that some 8-hydroxy-benzpyrene is produced on strongly heating the X_2/alumina adsorbate *in vacuo*.

The structural formulas of the various compounds and their respective transformations as inferred from their properties may be summarized as shown in Fig. 5.

to alumina and has the same appearance as the fluorescence of the salt of 8-hydroxy-benzpyrene in strong alkali. This indicates that it forms a salt with the basic aluminum oxide. From such an ionic adsorption it can be expected that the bonds of the benzpyrenyl residue itself, which control the fluorescence phenomena, are strongly affected. On the other hand, in F_1 it is the group R_1 (which does not belong directly to the benzpyrenyl residue) with acid properties that is strongly adsorbed to the basic alumina. The sky-blue fluorescence of this adsorbate, which according to Fig. 3 of our earlier paper (32) extends farther to the violet than the yellow-green fluorescence of the adsorbate of F_2, indicates that the original blue fluorescence of the benzpyrenyl residue is less affected than in F_2, although the band structure is suppressed. On the acid silica 8-hydroxy-benzpyrene

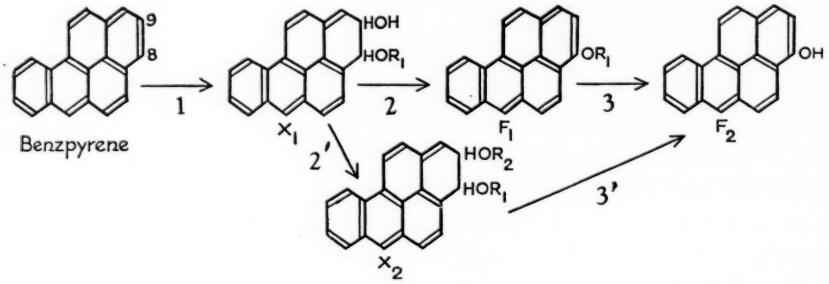


FIG. 5.—The various stages in the metabolic conversion of benzpyrene.

Although extensive use has been made of the fluorescence spectrograms (32, Fig. 3) of the various metabolites, their poor specificity makes them less reliable for chemical deductions than the absorption spectra in the near ultraviolet. They may be taken as a guide in the search for benzpyrene derivatives, but their indications must always be checked by other properties of the various metabolites. In experiments made under comparable conditions the intensity of the fluorescence spectra may be used for approximate estimates of the amount of a metabolite (*e. g.*, in Fig. 2), but under different conditions quenching effects, and especially the absorption of ultraviolet light by unknown impurities, may obscure these relationships completely.

However, one characteristic property of the fluorescence spectra of the adsorbates of the various metabolites with alumina and silica can be used as additional evidence of the suggested formulas. The fact that the fluorescence spectra of the two X-derivatives are identical with those of their respective solutions in organic solvents shows that the fluorescent benzpyrenyl group of the conjugates is not changed by the forces of adsorption. Hence it is likely that in adsorbed X_1 and X_2 the groups R_1 and R_2 are fixed to the adsorbing surface and that the fluorescent benzpyrenyl tails are free. The yellow-green fluorescence spectrum of F_2 shows no bands when fixed is not adsorbed, but F_1 is adsorbed weakly at its R_1-group by Van der Waals forces. Hence the fluorescence spectrum of F_1 adsorbed to silica is the same as that in organic solution owing to the unaffected benzpyrenyl tail.

One property of the absorption and fluorescence spectra is of some physical interest. It was observed with benzpyrene and all its metabolites, that the last violet, relatively sharp, absorption band standing at a position that is conspicuously detached from the ultraviolet bands, marks always the short wave length end of the fluorescence spectrum. This indicates a kind of resonance phenomenon.

B. Physiological Chemistry

The chief information about the sequence of events during the metabolic conversion of benzpyrene can best be inferred from its excretion via liver, bile, and small and large intestine. From these experiments it follows as a general rule that the quicker the X-metabolites travel through the lumen of the various cavities, and the shorter their contact with the respective walls, the greater is the relative amount of X_1. These conditions are optimal if a relatively large amount of a finely dispersed benzpyrene colloid is inoculated intravenously into a small animal. The X-derivatives are produced in the liver cells and rapidly carried away through the bile capillaries.

Hence X_1 almost exclusively is found in the liver tissue. In the gall bladder, which empties intermittently into the duodenum, temporary stagnations occur and favor the production of X_2. The small intestine, with its extended surface, increases still more the relative amount of X_2, which accumulates in the walls. The remaining X_1 in the lumen of the small intestine passes through the ileocecal valve into the cecum, where it is dehydrated to F_1. Whether this reaction is due to a reduction of pH, as in the experiments *in vitro,* or to the action of bacteria or ferments is not yet established.

The excretion of F_2 in the feces needs some comment. It increases in relation to F_1 when successive small portions are tested. This is not because F_1 is converted into F_2 but because the supply of X_1, at first abundant, soon decreases, whereas the supply of X_2, which is slowly detached from the wall of the small intestine, is longer maintained. Hence the origin of F_2 in the later stages of the excretion is probably due to a direct transformation of the accumulated X_2 to F_2 without passing through the X_1- or F_1-stages.

In addition there may occur a direct transformation of some F_1 to F_2 by analogy with the reaction of Boyland and Levi (7), who converted anthrylglucuronic acid to α-anthrol *in vitro.*

These phenomena show that X_1 is the first product of the metabolic conversion of benzpyrene that can be isolated. X_1 changes into F_1 by dehydration, or it is fixed to the tissue of the walls as X_2, an addition product of higher molecular weight. It is likely, but not yet established by direct experiments, that X_2 is directly transformed to F_2, which is excreted. These sequences are depicted in the structural formulas of Fig. 5. Only the transformation of X_1 to F_1 has as yet been effected *in vitro* under conditions comparable with those prevailing in the living animal.

The metabolic phenomena in other tissues are in agreement with those occurring in the digestive system, except that the transformation of X-derivatives into F-derivatives is less clear. In the kidney cortex and lung X_1 and X_2 were found in such proportion that the primary production of X_1 can be inferred. In the milk taken from suckling mice only X_1 was found, which indicates that the metabolite was quickly removed from the mammary glands with the milk. But from the skin and subcutaneous tissue, where X-derivatives persisted over long periods, X_2 almost exclusively was recovered. Finally, in the plasma, after the common bile duct had been cut, X_2 persisted for several days and the persisting blue fluorescence of the stomach, small and large intestine, kidney, lung, and liver, which is produced by this blue-fluorescent plasma, was characteristically of the X_2 type. All tissues, before extraction, show the X_2 fluorescence spectrum, even the liver and small intestine, which contain X_1 in abundance, because the X_2-bearing walls and cells themselves absorb chiefly the ultraviolet radiation. The appearance of F_1 in the stomachs of young mice suckling inoculated mothers is probably due to the conversion by hydrochloric acid of X_1 to F_1, as in the experiments *in vitro.* It will be recalled that in rabbit urine after its storage for a few days F_1 was found, whereas the kidney of this rabbit showed F_2. This difference is an indication that only X_1 is passed with the urine, and that X_2 is retained in the tissues of the kidney; it explains the fact that only small amounts of benzpyrene metabolites are excreted in the urine; and it is also in agreement with the evidence that no X-derivatives are absorbed from the small intestine into the blood stream because, on passage through the wall, X_1 is transformed into X_2, which is retained in the cells.

Only few quantitative data on the rate of metabolism of benzpyrene have been reported in this paper, and detailed experiments on the influence of various conditions are in preparation. However, it is apparent at the present stage of the study that the sequence of the various stages of the metabolic conversion as shown in Fig. 5 accounts almost quantitatively for passage of the hydrocarbon through the mouse. This is especially evident in the experiment reported (*3. Small intestine,* page 109), which was most favorable for estimation of the first metabolite X_1 with a yield of more than 70 per cent, which could be recovered from the liver and small intestine. This is certainly a lower limit, since even under these conditions some X_1 appeared in the kidney and was discarded, and some X_2 that could not be extracted with acetone was fixed in the wall of the small intestine.

The total yields of the F-metabolites (Table I) were distributed over a rather wide range, between 13 and 60 per cent of the metabolized benzpyrene. The estimations had to be made with various portions of the feces collected successively, and the errors due to limitations of the analytical method may accumulate. However, with respect to the order of magnitude the results agree with those inferred from the estimation of X_1, that a large proportion of the benzpyrene is removed from the body of the mouse in the form of the F-metabolites. Benzpyrene painted on the skin of mice is excreted in the form of F-metabolites, with a yield of about 50 per cent within 48 hours.

Stress has been laid on the puzzling discrepancy between the disappearance of benzpyrene from the peritoneal cavity and the appearance of F-metabolites in the feces (Fig. 1). The disappearance follows a reaction of the first order and the excretion one of zero order. Berenblum and Schoental (4) inferred

from the rate of disappearance according to a reaction of the first order, that the limiting factor controlling this rate is the diffusion from the site of injection into the blood. On the other hand, Berenblum and Schoental (4) established that the benzpyrene content of the blood remains almost constant from 1 hour up to 5 days after the intraperitoneal inoculation of 10 mgm. of benzpyrene, although the residual benzpyrene in the mouse had dropped to 2 mgm. after 5 days. This is consistent with a reaction of zero order for the excretion of F-metabolites in our Experiment 6 (Table I), which is controlled by the benzpyrene content of the blood. Until the results of further investigation of this strange discrepancy are known it must be concluded that there exists a second way, as yet unrecognized, by which the hydrocarbon can disappear from the peritoneum, in addition to its metabolism via X_1 to 8-hydroxy-benzpyrene.

Some evidence in favor of such a different mode of metabolism of benzpyrene is provided by the older experiments of Reggiani, Dansi, and Morelli (29), who studied the absorption spectra of extracts from rabbit bile and from livers and lungs of rats after chromatographic purification. These authors were unable to obtain any specific absorption spectra except that of benzpyrene itself when the organs were extracted a few hours after the intravenous injection of benzpyrene colloid. In order to accumulate an expected metabolite over longer periods they injected intravenously 4 mgm. benzpyrene in 0.2 cc. of an essential oil (pine oil, camphorated oil, and lecithin) into rats. The benzpyrene was immediately deposited in the lung capillaries and disappeared very slowly. After 15 days the rats were killed and the livers subjected to alkaline hydrolysis. The absorption spectra of the purified extracts from the unsaponifiable fraction was of the benzpyrene type, with an average displacement of the maxima by about 10 mμ towards the red. The corresponding extracts from the lungs showed the usual benzpyrene absorption spectrum.

The absorption spectra recorded for the liver extracts by these authors are unlike any of our X_1, X_2, F_1, or F_2 absorption spectra, but similar to that of 3,4-benzpyrene-5-carbamido-horse serum albumen, which was synthesized by Creech and Jones (13).

The mode of production of the X_1 in the cells of the living animal by "perhydroxylation" of benzpyrene is not yet known. It is probably due to the action of a peroxidase. However, it is significant that in the liver cells, as well as in those of the other tissues where X_1 is produced, benzpyrene has the opportunity of being dissolved in water. In the liver cells this is due to the presence of cholic acids and their derivatives; in the cells of the other tissues where X_1 is produced locally benzpyrene can be transformed into a water-soluble form by interaction with purine bodies (10), which comprise the dominant groups of the nucleotides.

The conclusions reached in this section are based almost exclusively on experiments with mice, which allow the quick recovery of the intermediate metabolites X_1, X_2, and F_1. Rats, which proved so valuable in the experiments especially of Chalmers and of Berenblum directed to establishing the end product of the metabolism, 8-hydroxy-benzpyrene, were used only occasionally; the fluorescence and absorption spectra of the various metabolites were identical with those of mice. The evidence from experiments with rabbits was considered only in 3 cases: (a) the appearance of F_2 in the tissue of the kidney cortex and of F_1 in the urine after storage without formol; (b) the occasional appearance of F_2 in fresh lungs; (c) the stability of X_1 in extracts of lungs even after 4 years. Although the fluorescence and absorption spectra of X_1, X_2, F_1, and F_2 from rabbits are the same as those of the corresponding derivatives from mice, it is certain that the metabolism in rabbits is different. Berenblum and Schoental (5) found in rabbits after death the 5,10-quinone as well as the 5,8-quinone, and according to Boyland and Levi (6) the optical rotations of 1,2-dihydroxy-1,2-dihydro-anthracene excreted from rats and rabbits are different.

C. Biological Considerations

The main purpose of this study was to search for a relation between the metabolic conversion of benzpyrene and its carcinogenic activity, and particularly to discover whether it is the hydrocarbon itself that induces the change from a normal to an abnormal cell, or whether the activity is due to a locally produced derivative. The general opinion is in favor of the activity of the hydrocarbon itself. This is in agreement with the fact that the final phenolic products of excretion of carcinogenic hydrocarbons, at any rate in the case of 1,2,5,6-dibenzanthracene (16, 19) and 3,4-benzpyrene (1, 5), are considerably less carcinogenic than their parent hydrocarbons. This transformation was therefore considered as a protective detoxicating mechanism.

However, the new result that 8-hydroxy-benzpyrene is not a direct product of transformation from the hydrocarbon by a catalyzed oxidation, but that a number of intermediate derivatives are passed through, opens the way for a detailed discussion of the carcinogenic action. Since it has never been possible to detect in a tumor any traces of the chemical agent by which it was produced, there is only one promising approach to the problem: the various stages of the metabolic conversion must be studied quantitatively

under different conditions and the results compared statistically with the incidence of tumors under the same conditions. Work on these lines is in progress, especially with respect to the carcinogenic activity of the various intermediate metabolites themselves,[3] which have been prepared from extracts from mice.

At the present stage we are able to consider only two significant phenomena, which have been reported earlier. The first (Fig. 2) was observed on the skin: after painting benzpyrene on both flanks of mice, one side having been "sensitized" by prepainting with croton oil, the blue X_2-fluorescence on the unsensitized (control) side was stronger and disappeared more slowly than that on the sensitized side. The same difference was apparent with the painted hydrocarbon itself, which disappeared more slowly on the control side. The biological counterpart to this experiment was carried out by Mottram (26), who established that prepainting of the skin with croton oil increased the crop of tumors.

The second phenomenon is concerned with the subcutaneous tissue. Benzpyrene dissolved in sesame oil was injected in equal amounts into one flank of a mouse, and benzpyrene dissolved in mouse fat, into the other. After 3 days there was a much stronger blue X_2 fluorescence remaining in the tissue where mouse fat had been used than on the sesame oil side. This agrees well with the results of Dickens (14), who studied the carcinogenesis of benzpyrene in various lipoid solvents. Benzpyrene dissolved in mouse fat persisted particularly long at the site of the subcutaneous injection.

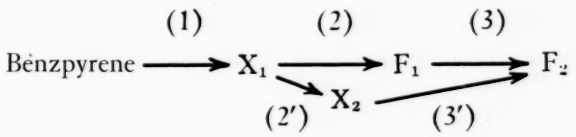
However, according to Peacock and Beck (28) and to Dickens and Weil-Malherbe (15), the carcinogenic activity of benzpyrene in mouse fat is very low, and the paradoxical result of the two experiments (painted and subcutaneously injected benzpyrene) is that the hydrocarbon is more quickly absorbed and the X_2 fluorescence less strong on just that side of the mouse where it is known that the incidence of tumors is high.

These two biological phenomena can be discussed in connection with the various chemical stages of the metabolism as shown in Fig. 5. X_1 disappears by two competing routes: either by direct transformation into F_1, which is quickly excreted, or by fixation to the tissues as X_2. The persistence of X_2 when the carcinogenic activity is low seems to indicate that the fixation as X_2 acts as a detoxication mechanism.

However, since the radicals R_1 and R_2 in X_1, X_2, and F_1 are derived from the cells inside which the metabolic conversion occurs,[4] these cells themselves must be considered as essential members of the chemical mechanism. Furthermore, the mobilization of the enzymes and catalysts that promote the various transformations must be taken into account. From this point of view it is possible to devise several schematic models to depict the fate of the interacting cells as a consequence of the chemical reactions. They show that after the intracellular metabolism of benzpyrene has taken place, some abnormal cells are left that have suffered a loss of the groups R_1, R_2, or both. These may act as the mother cells for tumor genesis. However, although these models explain the paradoxical phenomena on the painted skin and in the inoculated subcutaneous tissue discussed above, it is premature to consider them in detail until many more quantitative biological experiments are available.

SUMMARY

The metabolic conversion of 3,4-benzpyrene in mice passes through a number of stages, symbolized by X_1, X_2, F_1, and F_2 according to the following abbreviated sequence:



The various metabolites are characterized by their fluorescence and absorption spectra and by their chemical and chromatographic properties, which suggest their chemical constitutions. Among the derivatives

$X_1 = 8(OR_1)\text{-}9(OH)\text{-}8,9\text{-dihydro-}3,4\text{-benzpyrene}$
$X_2 = 8(OR_1)\text{-}9(OR_2)\text{-}8,9\text{-dihydro-}3,4\text{-benzpyrene}$
and $F_1 = 8(OR_1)\text{-}3,4\text{-benzpyrene}$

have not been described previously, whereas F_2 is the known end product of the metabolism, 8-hydroxy-3,4-benzpyrene. After an intravenous inoculation of a finely dispersed colloid the metabolism of 3,4-benzpyrene follows the sequence above in an approximately quantitative manner. The nature of the radicals R_1 and R_2 is not yet established, but they are derived from the structure of the cells with which the parent hydrocarbon and X_1 come into contact. The steps 1 and 2′ occur *in vivo* only, whereas 2 can be reproduced *in vitro* by a mild chemical reaction at room temperature, and 3 and 3′ by stronger agents at elevated temperature.

[3] Berenblum (1) recently reported a high carcinogenic potency for 8-(OCH_3)-benzpyrene, a substance closely related to 8-(OR_1)-benzpyrene; *i.e.*, F_1.

[4] A connection between R_1 and R_2 and the sulphur metabolism of the cells may be inferred by analogy from the evidence that Boyland and Levi (8) recovered 1-anthrylmercapturic acid after the metabolic conversion of anthracene.

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Effects of Massive Doses of Methylcholanthrene on Epidermal Carcinogenesis*

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In the production of carcinoma of the skin in mice methylcholanthrene is usually applied several times a week in a 0.3 to 0.6 per cent solution in benzene. Its toxicity is often manifest locally by necrosis of the epidermal cells and ulceration; systemically by a decrease in body weight. These changes are more frequently seen in animals subjected to larger doses of methylcholanthrene. According to one concept of carcinogenesis by methylcholanthrene, the initial action of the compound on epidermal cells is that of a toxic agent, which damages the cells and produces variable degrees of necrosis (1, 15). Subsequently the regenerating and proliferating epidermis seems to develop a tolerance to the toxic action. The present series of experiments was undertaken to determine whether the application of massive doses of methylcholanthrene to the skin would result in domination of the toxic local and systemic effects of the carcinogen and a diminution in its ability to produce cancer.

In a small group of mice Cramer and Stowell (5) applied 1.0 per cent methylcholanthrene to the skin 3 times a week, and showed that the increase in the rate of occurrence of the tumors was not proportional to the dosage of methylcholanthrene. In the present experiments the mice received up to 3 times as much methylcholanthrene as the largest amounts used by Cramer and Stowell, and 200 times the minimal amount they employed (4). The use of such large amounts afforded an opportunity to study the effects of the methylcholanthrene and its detoxification products upon the viscera.

MATERIALS AND METHODS

Except for the dose of methylcholanthrene employed, the conditions of the experiment were comparable to those of previous experiments (2-5) performed at the Barnard Hospital. The Swiss mice were obtained from Tumblebrook Farms, fed a diet of Rockland pellets, and kept in an air-conditioned room. Female mice 8 weeks of age, with an average weight of 18 gm., were divided into 5 groups. Three groups of 50 mice each were painted on a large area of the back with a solution of 1.0 per cent methylcholanthrene in analytical grade benzene. Because of rapid evaporation of the solvent a saturated solution of methylcholanthrene in benzene produces a loose, yellow deposit of solid methylcholanthrene on the skin, which is readily removed before it can be absorbed. It was thought that more methylcholanthrene could be introduced into the skin by frequent painting with a 1 per cent than with a saturated solution. Therefore 1 group received 3 applications, another 6, and a third 9 applications weekly of a 1 per cent solution. A fourth group of 30 control mice was painted with pure benzene 9 times each week. All paintings were stopped after 14 weeks. The solutions were applied to the back in a uniform manner, with a single stroke of a No. 4 camel's hair brush. Precautions were taken to prevent the inhalation of benzene fumes by the mice. A fifth group of 18 control mice received no paintings.

The mice were carefully examined each week and notes made regarding their general appearance, epilation, ulceration, swellings, papillomas, and malignant tumors. After the development of carcinomas the animals were killed with ether and complete necropsies were performed. The tissues were fixed in Bouin, Zenker-formol, and 10 per cent formalin, dehydrated in ethyl alcohol, cleared in chloroform, and embedded in paraffin. One or more sections were made through each skin tumor suspected of being malignant. The tissues examined microscopically included adrenal, brain, duodenum, esophagus, fallopian tube, femur, heart, kidney, liver, lumbar vertebral body, lung, lymph node, ovary, pancreas, parathyroid, pituitary, rib, salivary gland, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, thymus, thyroid, and trachea. In most instances serial sections were prepared of the adrenal, ovary, pituitary, thyroid, and parathyroid.

The benzene-painted and the unpainted control animals were killed in 3 groups, corresponding to the age of the first, median, and last experimental animals to be killed with tumors. The necropsies and microscopic examinations of the control mice were carried out in the same manner as for the mice painted with methylcholanthrene.

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RESULTS

Although epilation is not a frequent finding in mice painted with moderate amounts of pure benzene 3 times a week, in the benzene control mice painted 9 times a week 80 per cent of the animals showed varying degrees of epilation after 25 paintings. All the mice painted with methylcholanthrene in benzene showed some epilation, which occurred sooner and lasted longer in those painted 9 times weekly. A fourth of the mice receiving the largest amounts of methylcholanthrene lost some of the vision in one or both eyes, and most of their hair, except over the distal part of the extremities and the anterior and ventral surface of the head. The corneal opacity, which developed chiefly between the sixth and 14th weeks, was presumed to result from contact with the methylcholanthrene, since none of the benzene control mice showed similar changes. A few animals developed suppurative blepharitis, and several developed papillomas and carcinomas on the eyelids. During the course of the paintings ulcerations of the skin developed in 60, 80, and 75 per cent of the mice painted 3, 6, and 9 times a week. Some of those receiving large amounts of carcinogen showed a decrease in body weight, and in several instances it was necessary to suspend treatment for a few days to prolong life. The general health of the animals appeared to improve following the cessation of paintings in the 14th week of the experiment.

The mortality rates from enteric infections and accidents during the first 3 months of the experiment were 26, 18, 20, 37, and 25 per cent for the mice painted 3, 6, and 9 times weekly and for the benzene and unpainted control animals. All surviving mice painted with the carcinogen developed malignant tumors of the skin. Occasionally it was not possible to preserve the internal viscera for microscopic sections because the animals were found dead.

The incidence of malignant epithelial tumors in the 3 groups is given in Table I and represented graphically in Fig. 1. The microscopic sections were graded according to the degree of anaplasia of each tumor, but no significant mean difference among the 3 groups was found. An example of a moderately anaplastic carcinoma, which arose on the ear of a mouse painted with massive doses of methylcholanthrene, is shown in Fig. 2. The mice receiving 9 paintings a week developed malignant tumors more rapidly, as shown by a mean induction time of 14.6 weeks compared with 16.6 and 16.9 weeks for the other groups. The mean number of tumors produced per mouse was similar for each group. Metastasis to lymph nodes was demonstrated by microscopic section in 2 animals.

Important pathologic changes were observed microscopically in the liver, kidneys, lungs, spleen, lymph nodes, and bone marrow. From a third to half of the animals painted with methylcholanthrene had acute or chronic inflammation of the liver. Collections of lymphocytes and mononuclear cells, which were found about the blood vessels within the livers of most control and experimental animals, were not considered significant. However, only 1 control animal painted with benzene had a perivascular infiltration containing numerous polymorphonuclear leukocytes. Such evidence of acute inflammation (Fig. 3) was a frequent observation in mice painted with the carcinogen, though it was only slightly more frequent in those treated with the largest dosage. Small foci of necrosis of hepatic cells were present in a few experimental animals. As shown in Fig. 4, the hepatic parenchyma in some mice, particularly in the periportal areas, was disrupted and irregularly replaced by fibrous tissue containing polymorphonuclear leukocytes, lymphocytes, mononuclear cells, fibroblasts, and reticulum cells. Bacterial stains on the sections did not reveal micro-organisms. In several instances the microscopic appearance resembled that of a granulomatous reaction of undetermined type. Myelopoiesis was present in the sinusoids of some livers. Only 3 specimens showed advanced fatty metamorphosis.

There were accumulations of polymorphonuclear leukocytes and lymphocytes about the muscularis mucosa of the stomach in a few experimental animals. In an occasional mouse there were small foci of acute inflammatory reaction about the pancreatic ducts or within the peripancreatic adipose tissue.

The kidneys showed several types of pathologic change. Both control and experimental animals showed variable numbers of lymphocytic and mononuclear cells about the large and medium-sized renal arteries and beneath the epithelium of the renal pelvis. Of more significance were areas within the cortex containing an increased amount of connective tissue and an infiltration with lymphocytes, mononuclear cells, and occasional polymorphonuclear leukocytes. There was some replacement of glomeruli and tubules by fibrous tissue. Bacteria could not be demonstrated by special stains. This inflammatory reaction, resembling a chronic pyelonephritis, was present in from a fifth to a half of the experimental animals and in 2 benzene control and 1 unpainted control mouse. One experimental animal had acute pyelonephritis with abscess formation and 2 had abscesses in the perirenal tissue. Statistical analysis showed that there was no difference in the mean age of mice with inflammation of the kidney and those in which it was absent.

Bronchopneumonia was present in many experimental mice, and absent in all control animals. Statistical analysis showed that age of mouse or dosage of carcinogen were not significant factors in the incidence of bronchopneumonia. In relatively few animals were

TABLE I: INCIDENCE OF MALIGNANT TUMORS IN MICE PAINTED 3, 6, AND 9 TIMES A WEEK WITH 1.0 PER CENT METHYLCHOLANTHRENE IN BENZENE

	3 × weekly	6 × weekly	9 × weekly
First mouse developed carcinoma (in weeks)	10	7	8
50% of mice developed carcinoma (in weeks)	16	17	14
100% of mice developed carcinoma (in weeks)	28	26	20
Mean induction time for carcinomas (in weeks)	16.9	16.6	14.6
Average no. of tumors per mouse	2.4	2.3	2.4
Percentage epidermoid carcinomas	98	94	97
Percentage spindle cell carcinomas and sarcomas	2	6	3
No. of effectual mice	37	41	40

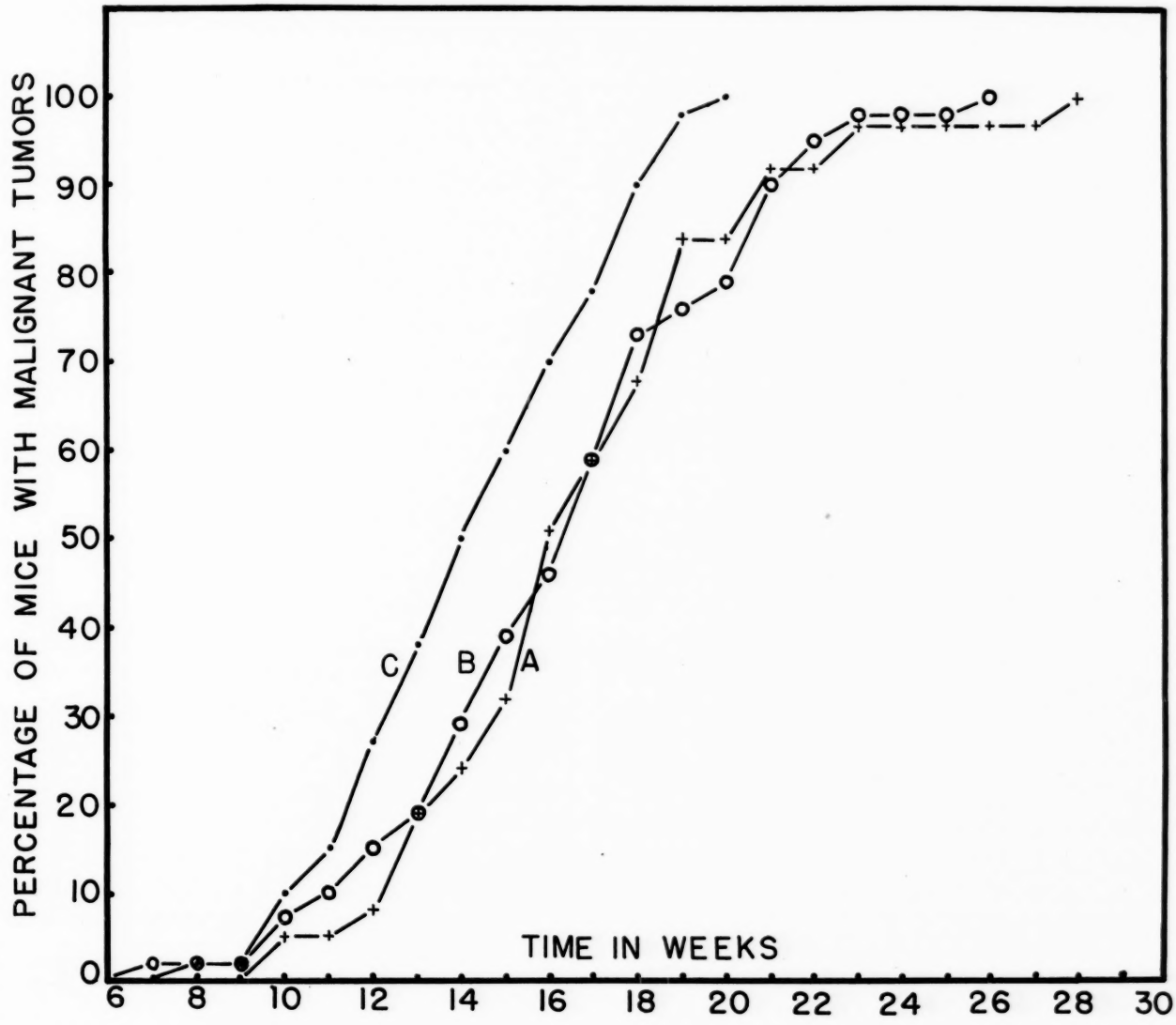
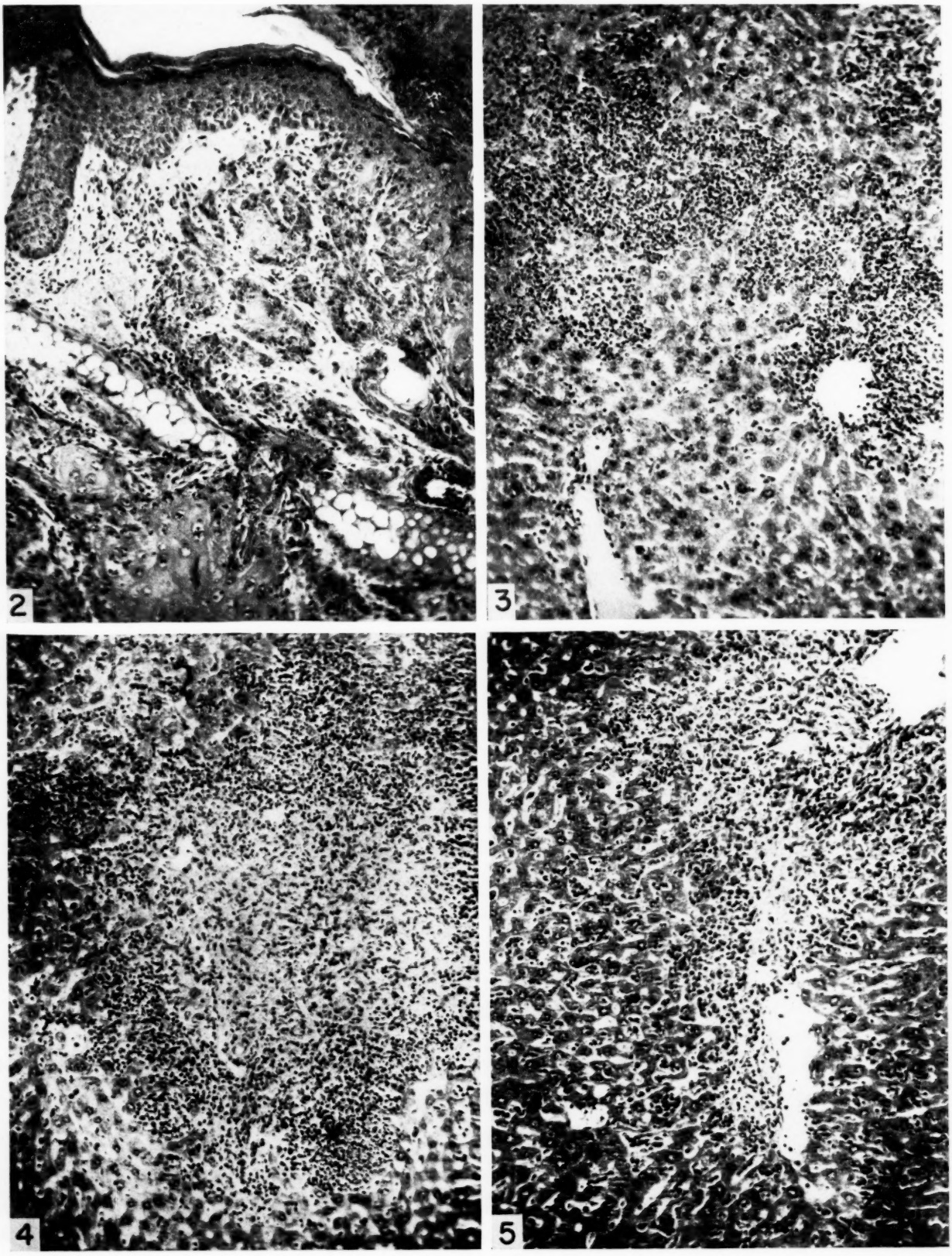


FIG. 1.—Incidence of malignant tumors in mice painted with 1.0 per cent methylcholanthrene in benzene 3 (A), 6 (B), and 9 (C) times a week for 14 weeks.

the lungs the only site of inflammatory reaction. A few experimental animals showed considerably more perivascular infiltration with lymphocytes and mononuclear cells than the control animals. From 15 to 33 per cent of the mice in the groups painted with methylcholanthrene and 1 benzene control animal had adenomas of the lung; one mouse had 32 adenomas in 1 section through 4 lobes. The mean ages at the time of death for the groups with adenomas was up to 2 weeks older than for the mice without adenomas. However, for the small number of animals involved there was no significant statistical correlation between the incidence or number of adenomas per section and the age of the mice or dose of carcinogen applied.

Some specimens of bone marrow, lymph nodes, and



FIGS. 2-5

spleen in the mice painted with pure benzene showed a slight hyperplasia. However, the bone marrow in half the experimental mice showed definite hyperplasia, chiefly of the myeloid series. In some mice there was decreased erythropoiesis. The spleen was enlarged and hyperplastic in a majority of the experimental mice, and myelopoiesis was often present. Often the hyperplasia was not associated with demonstrable inflammation elsewhere. It was found by statistical analysis that there was no significant correlation between the age of the mice and the incidence of hyperplasia of the spleen. Hyperplasia of the lymph nodes, however, was usually associated with inflammation. Occasional lymph nodes contained focal areas of necrosis.

Two mice painted 6 times weekly and 3 painted 9 times a week showed pathologic changes characteristic of leukemia (Fig. 5). The leukemia in 3 mice was of a myeloid, and in 2 of a lymphoid character. In the absence of observations on fresh blood, and transplantation studies, it was not possible to be certain that the extramedullary myelopoiesis and lymphopoiesis observed in some other experimental animals was not evidence of early leukemic or leukemoid changes.

Amyloid deposits were present in the livers of 2 experimental mice and in the kidney of another. Occasional foci of polymorphonuclear leukocytes and lymphocytes were present in the mesenteric adipose tissue.

Serial sections of the thyroid and parathyroid glands showed similar changes in both experimental and control mice. An occasional thyroid gland appeared slightly atrophic. About half of the experimental and control mice had enlarged thyroid acini containing granular, foamy, or vacuolated colloid that showed considerable tinctorial variation after staining with Heidenhain's azocarmine modification of Mallory's triple connective tissue stain. The epithelium lining these distended acini was sometimes flat and occasionally of a columnar or ciliated columnar type. Some of these atypical acini were located near the parathyroid glands. In about half of the experimental and control mice the cells of the parathyroids were enlarged and showed clumping of the chromatin, with prominent nucleoli. Such changes were not always uniformly distributed throughout all parts of the glands in the same mouse. Some of these parathyroid glands were increased in size. The changes in the thyroids and parathyroids were not uniformly present in the same animals and could not be readily correlated with other factors.

The incidence of significant inflammatory and hyperplastic lesions in some of the organs is given in Table II. The adrenals, brain, duodenum, esophagus, fallopian tube, heart, ovaries, pituitary, salivary glands, skeletal muscle, spinal cord, thymus, and trachea were rarely the site of significant pathologic change. In several mice the thymus was atrophic.

In summation, an attempt was made to classify each mouse as to whether the primary pathologic alteration, if any, was (a) inflammatory; (b) hyperplasia of myeloid, lymphoid, or erythroid elements; or (c) leukemic. The results of this interpretation, which was difficult in some instances, are shown in Table II.

DISCUSSION

Although it is possible to reduce the induction time for epidermoid carcinomas by applying larger amounts of methylcholanthrene to the skin of mice, the relation certainly does not follow a direct proportion (5). Under the conditions of this experiment, massive exposure of the skin did not result in sufficient toxic action to decrease the incidence of carcinomas. A threefold increase in the dose of carcinogen did not significantly alter the mean number of tumors elicited per animal or the degree of anaplasia of the tumors. These results do not conclusively support either theory of carcinogenesis: that methylcholanthrene directly stimulates the uncontrolled proliferation of epithelial

DESCRIPTION OF FIGURES 2 TO 5

FIG. 2.—Epidermoid carcinoma of skin of mouse after application of 1.0 per cent methylcholanthrene in benzene 9 times weekly for 12 weeks. One of the few tumors on the ear. The moderately anaplastic tumor extends through the cartilage. Hematoxylin and eosin. Mag. × 135.

FIG. 3.—Mouse liver after application of methylcholanthrene 9 times weekly for 14 weeks. Animal in poor health when killed. Liver shows a granulomatous inflammatory reaction, with infiltration of polymorphonuclear leukocytes, lymphocytes, mononuclear cells, and occasional giant cells, which were not seen in most instances of acute inflammatory reactions of the liver. This mouse also had inflammation of the kidney and wall of the stomach, and hyperplasia of the spleen. Hematoxylin and eosin. Mag. × 135.

FIG. 4.—Areas of fibrosis of liver containing large fibroblasts, surrounded by a zone of acute inflammation with many polymorphonuclear leukocytes and some lymphocytes. This same mouse had an area of myeloid infiltration in the liver, bronchopneumonia, chronic pyelonephritis, and hyperplasia of myeloid and lymphoid elements in the bone marrow and lymph nodes. Treatment was the same as that described in Fig. 3. Hematoxylin and eosin. Mag. × 135.

FIG. 5.—Liver of mouse painted 9 times weekly for 14 weeks and killed 21 weeks after first treatment. As part of a generalized leukemia, the liver contains leukemic cells of varied types in the myeloid series. Hematoxylin and eosin. Mag. × 135.

TABLE II: SYSTEMIC PATHOLOGIC CHANGE IN UNPAINTED CONTROL, BENZENE CONTROL, AND MICE PAINTED 3, 6, AND 9 TIMES WEEKLY WITH 1.0 PER CENT METHYLCHOLANTHRENE IN BENZENE

	Unpainted control	Benzene control	3 × weekly	6 × weekly	9 × weekly
Inflammation (in per cent)					
Liver	0	5	39	33	57
Kidney	8	10	24	19	50
Stomach	0	0	3	6	23
Pancreas	0	0	3	3	12
Lung	0	0	44	26	36
Hyperplastic changes (in per cent)					
Bone marrow	0	0	50	41	42
Spleen	0	5	76	70	57
Lymph nodes	0	5	7	22	32
Adenomas of lungs (in per cent)	0	5	32	33	15
Principal pathologic change (in per cent)					
Inflammation, including pneumonia	8	10	56	43	53
Pneumonia only	0	0	12	6	7
Hyperplasia	0	5	18	25	18
Leukemia	0	0	0	6	11
Normal	92	85	26	26	18
Effectual mice with complete necropsy	13	19	34	34	28
Mean age at death (in weeks)	25	25	26.3	26.0	24.0

cells; or that it exerts a toxic action, from which some surviving cells escape by adopting abnormal growth characteristics. It seems probable that the application of larger amounts of carcinogen than employed in these experiments would result in an increased mortality among the experimental animals.

Recently Glücksmann (7) has discussed the histogenesis of tumors in mice produced by carcinogenic hydrocarbons. In evaluating the differences between his own careful observations and those of previous workers, one must recognize the importance of the fact that he employed a different carcinogen of smaller carcinogenic potency, a different solvent, and a different method and frequency of application from most other investigators. Such variations in technic are known to affect the results materially.

The group of animals receiving the largest amounts of methylcholanthrene showed the most systemic toxic effects. Their general appearance, activity, incidence of corneal opacity, and generalized epilation were clinical evidence of poor general health. Since 10 animals were kept in each cage, considerable methylcholanthrene was always present in their environment. The smallest percentage of animals showing no pathologic changes in the viscera was in the group exposed to the most carcinogen. In this group inflammatory lesions were more frequent in all organs except the lungs. Statistically there was no significant correlation between the age of the mice and the incidence of inflammation in the liver, kidney, or lungs, or of hyperplasia of the spleen.

It is unfortunate that because of technical difficulties sections of the organs were not prepared before the conclusion of the experiment; hence the desirability of bacteriologic studies and observations on fresh blood was not realized in time. In the absence of such observations one is not justified in definitely stating the cause of the pathologic changes in the viscera, or their exact relationship to application of the carcinogen. Thus, as in most other reports on the systemic effects of carcinogenic compounds, these results are somewhat inconclusive. Comparison with the benzene and unpainted control animals does show that the methylcholanthrene either (a) directly produced inflammatory changes in organs such as the liver, kidney, and gastrointestinal tract where it is probably detoxified and excreted; or (b) lowered the resistance of the host so that such inflammation was more easily produced by infectious agents. It is also not unlikely that both mechanisms were operative in some instances. Furthermore, the methylcholanthrene either directly or indirectly elicited hyperplasia and extramedullary myelopoiesis and lymphopoiesis. Some of these changes were probably of preleukemic nature, since several cases of definite leukemia were recognized. Sections of organs were shown to several other pathologists, including Dr. Jacob Furth, who concurred in these opinions.

Some of the changes observed in the viscera were similar to those reported by other authors, chiefly following the injection of animals with carcinogens. The occurrence of leukemia and leukemoid states following exposure to chemical carcinogens has been reviewed by numerous investigators, including Kirsch-

baum (9) and Morton and Mider (11). Exhaustion of lymphoid tissue and pleural effusion were reported by Picard and Laduron (12, 13); fatty degeneration, dissociation of liver cell cords, periportal proliferation, and cirrhosis by Polson (14), Claude (1), and Goerner and Goerner (8). After a single injection of benzpyrene, Leuchtenberger and Sicher (10) observed periarteritis nodosa, diffuse myocarditis, and extensive mesenchymal proliferation of an inflammatory nature. No instance of true periarteritis nodosa or diffuse myocarditis was found in the present series of animals.

Experiments that are in progress by Van Dyke (16, 17) show similar but more extensive changes in the thyroid and parathyroid glands of mice painted with methylcholanthrene, though his mice were of a different strain and treated under different conditions; he has interpreted his observations as alterations in the ultimobranchial bodies. Dunn (6) has reported ciliated acinic epithelium of the thyroid in normal mice, and reviewed the observations of several other investigators on degenerative changes in this gland. The nature and etiology of such alterations need additional investigation. The mice in our experiments did not show sufficient qualitative or quantitative difference between the two control and experimental groups painted with large amounts of methylcholanthrene to justify the assumption that the degenerative lesions in the thyroid or the hypertrophy and hyperplasia of the parathyroids were directly related to the treatment with methylcholanthrene.

SUMMARY AND CONCLUSIONS

Three groups of approximately 50 Swiss mice each were painted on the back with solutions of 1.0 per cent methylcholanthrene 3, 6, and 9 times a week, while 2 other groups were used as unpainted controls and controls painted with pure benzene. Such tremendous amounts of methylcholanthrene produced malignant tumors a little more rapidly than smaller doses. As systemic effects of exposure to the methylcholanthrene the mice showed increased incidences of (a) inflammation in the liver, kidney, and lungs; (b) hyperplasia of the bone marrow, spleen, and lymph nodes with extramedullary myelopoiesis, lymphopoiesis and erythropoiesis; and (c) leukemia. It is not known whether these changes were brought about directly by the methylcholanthrene and its detoxification products, or indirectly through lowered resistance of the host to bacterial or viral agents.

These results do not conclusively support either the theory that chemical carcinogens act directly by stimulating uncontrolled cell proliferation; or by a toxic action from which some surviving cells escape by adopting abnormal growth characteristics.

Some thyroid glands had atypical acini and some parathyroids were hypertrophic and hyperplastic. These experiments do not support the idea that such changes are primarily caused by the methylcholanthrene.

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Neutralization of Inhibition of Tumor Growth

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The antagonism between compounds promoting and inhibiting growth has received increasing attention in the past few years. Recently, as the result of a series of investigations with growing animals and micro-organisms, the findings of Woods (8) on the interference of *p*-aminobenzoic acid with sulfanilamide action have been extended by Woolley (9). In the studies to be described here it has been possible to demonstrate antagonisms between structurally related, and in some cases unrelated, substances by using tumor growth and its inhibition to show these relationships.

Assay by a method involving the growth of transplanted tumors in mice (2) has shown that various substances, such as inositol (3), *Lactobacillus casei* factor (4), and xanthopterin (5) are effective in inhibiting the growth of tumors. However, among the substances and extracts tested none was found that stimulated tumor growth. It therefore appears that the transplants were probably growing at an optimal rate. A method was then devised to demonstrate the possible growth-stimulating properties of various substances by measuring their interference with tumor growth inhibitors. This was accomplished by repressing the growth rate of a transplant by injecting mice intravenously with a suitable dose of a proved inhibitor, and at the same time with a suitable dose of the substance or extract to be tested for antagonistic activity, the amount of inhibitor neutralized being a measure of the degree of interference.

Method

Groups of 7 or 8 female Rockland mice bearing transplanted tumors of similar size (sarcoma 180, 7 to 10 days after transplantation) and maintained on a normal diet were selected in a manner previously described (2). Each mouse of a group was injected intravenously twice a day for 2 consecutive days with the substance or mixture of substances under test. Forty-eight hours after the first injection the animals were killed with ether, the tumors removed, freed from surrounding tissue, and weighed. Probability ratios were calculated as to inhibition and interference with inhibition.

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Results

In Table I results are given for the effectiveness of *p*-aminobenzoic acid in overcoming the inhibition of tumor growth by inositol. From these experiments it appears that when equal amounts, 100γ, are injected, the inhibitory effect of inositol is almost completely neutralized. If 50γ of *p*-aminobenzoic acid

Table I: Effect on Tumor Growth of 4 Intravenous Injections of Inositol and Mixtures of Inositol and *p*-Aminobenzoic Acid

	Mean terminal tumor wt., mgm.	Probability ratios	
		Inhibition	Interference
Experiment No. 1			
Saline control	765		
50γ Inositol	548	4.5	
100γ Inositol	312	9.6	
100γ Inositol and 100γ *p*-aminobenzoic acid	687	1.3	6.9
Experiment No. 2			
Saline control	834		
100γ Inositol	413	5.4	
100γ Inositol and 50γ *p*-aminobenzoic acid	581	3.3	4.7

are injected with the 100γ of inositol there is partial neutralization of the inositol and the response is that of approximately 50γ of inositol.

In order to gain further information concerning the possible mechanism of the antagonistic action of *p*-aminobenzoic acid against inositol, a number of substances structurally related or unrelated to *p*-aminobenzoic acid were tested with inositol. In Table II 3 experiments are given in which *para-, meta-,* and *ortho*-aminobenzoic acid, niacinamide, pyridoxin, thiamine, and leucopterin were tested in combination with inositol.

It is evident from Table II that *p*-aminobenzoic acid and pyridoxin neutralized the inositol inhibition completely if equal quantities of each were administered. If smaller amounts of pyridoxin were injected, the surplus in inositol was as evident as in the experiment with *p*-aminobenzoic acid (Table I, Experiment 2).

In contrast to these findings, *meta-* and *ortho*-aminobenzoic acid, niacinamide, thiamine, and leucopterin

interfered with the inositol inhibition to a considerably lesser extent.

Biotin has been shown previously not to inhibit tumor growth (3), whereas *d*-desthiobiotin (Table III) is effective as an inhibitor. The degree of inhibition depends upon the dose injected, maximal inhibition being obtained with 5 to 10γ of desthiobiotin, depending on the growth rate of the transplants.

In Table IV is shown the antagonistic relationship between desthiobiotin and biotin. The neutralization of inhibition was effected by 5γ of biotin,[1] which, by itself, was without result.

An avidin "concentrate" [2] was tested, and the results (Table V) demonstrate that it is a potent inhibitor. The maximal inhibition was obtained at the 40γ level, and significant inhibition was produced even with 4γ.

When the avidin concentrate was tested in conjunction with desthiobiotin,[1] interference occurred. The results (Table VI) show that these two inhibitors can neutralize one another. Desthiobiotin at the 5γ level was neutralized by a 10γ dose of the avidin concentrate. However, larger doses of the avidin concentrate, 20γ and 40γ, overcame the antagonistic effect of the 5γ of desthiobiotin with a resultant inhibition of tumor growth.

TABLE II: EFFECT ON TUMOR GROWTH OF 4 INTRAVENOUS INJECTIONS OF INOSITOL AND A MIXTURE OF 100γ INOSITOL AND TEST SUBSTANCES

	Mean terminal tumor wt., mgm.	Probability ratios: Inhibition	Probability ratios: Interference
Experiment No. 1			
Saline control	733		
50γ Inositol	495	2.7	
100γ Inositol	380	4.8	
100γ Inositol and 100γ *p*-aminobenzoic acid	751	—0.2	7.2
100γ Inositol and 500γ *m*-aminobenzoic acid	435	4.1	1.0
100γ Inositol and 500γ *o*-aminobenzoic acid	585	2.1	5.5
100γ Inositol and 100γ niacinamide	385	5.0	0.1
100γ Inositol and 250γ niacinamide	527	2.5	2.5
Experiment No. 2			
Saline control	711		
50γ Inositol	524	2.8	
100γ Inositol	343	7.6	
100γ Inositol and 100γ thiamine	395	6.1	1.6
100γ Inositol and 250γ thiamine	491	3.7	3.3
100γ Inositol and 50γ pyridoxine	498	2.7	2.5
100γ Inositol and 100γ pyridoxine	691	0.3	5.9
100γ Inositol and 250γ pyridoxine	747	—0.6	9.5
Experiment No. 3			
Saline control	867		
50γ Inositol	671	3.0	
100γ Inositol	501	5.3	
100γ Inositol and 5γ leucopterin	503	5.8	0.1
100γ Inositol and 50γ leucopterin	607	3.0	1.4
50γ Inositol and 50γ leucopterin	726	2.0	1.1

TABLE III: EFFECT ON TUMOR GROWTH OF 4 INTRAVENOUS INJECTIONS OF *d*-DESTHIOBIOTIN

	Mean terminal tumor wt., mgm.	Probability ratio Inhibition
Saline control	927	
0.2γ *d*-desthiobiotin	873	0.7
1.0γ *d*-desthiobiotin	643	3.2
5.0γ *d*-desthiobiotin	458	5.8
10.0γ *d*-desthiobiotin	441	6.1
50.0γ *d*-desthiobiotin	462	5.6

TABLE IV: EFFECT ON TUMOR GROWTH OF 4 INTRAVENOUS INJECTIONS OF BIOTIN, *d*-DESTHIOBIOTIN, AND A MIXTURE OF BIOTIN AND *d*-DESTHIOBIOTIN

	Mean terminal tumor wt., mgm.	Probability ratios: Inhibition	Probability ratios: Interference
Saline control	1,050		
0.2γ biotin	970	0.7	
1.0γ biotin	976	0.7	
5.0γ biotin	1,037	0.1	
0.2γ *d*-desthiobiotin	869	1.7	
1.0γ *d*-desthiobiotin	838	1.9	
5.0γ *d*-desthiobiotin	691	3.4	
5γ *d*-desthiobiotin and 1.0γ biotin	674	3.2	0.2
5γ *d*-desthiobiotin and 5.0γ biotin	1,069	0.1	3.5

TABLE V: EFFECT ON TUMOR GROWTH OF 4 INTRAVENOUS INJECTIONS OF AN "AVIDIN CONCENTRATE"

	Mean terminal tumor wt., mgm.	Probability ratio Inhibition
Saline control	888	
2γ "avidin concentrate"	775	1.5
4γ "avidin concentrate"	565	5.6
20γ "avidin concentrate"	573	5.3
40γ "avidin concentrate"	415	7.4
200γ "avidin concentrate"	428	7.0

DISCUSSION

With a rapidly growing tumor for the detection of inhibitors of tumor growth in a 48 hour test period, a method described has now been adapted to demon-

[1] No significant differences were seen whether the substances were injected separately at short intervals or mixed in the syringe just prior to injection.

[2] About 12 per cent pure.

strate that the action of tumor-growth inhibitors can be effectively neutralized. Even though we are dealing with a complex biological phenomenon, the biotin-desthiobiotin stimulation-inhibition relationship shown by Dittmer and his associates (1) and Lilly and Leonian (6) for micro-organisms operates also under the conditions of our methods. Desthiobiotin is the tumor-growth inhibitor, and biotin its antagonist. Although in our assay approximately equal amounts of each will demonstrate this antagonism, in the work with *L. casei* (1, 6) the desthiobiotin exerted its inhibition when present at several thousand times the level of biotin.

TABLE VI: EFFECT ON TUMOR GROWTH OF 4 INTRAVENOUS INJECTIONS OF A MIXTURE OF *d*-DESTHIOBIOTIN AND "AVIDIN CONCENTRATE"

	Mean terminal tumor wt., mgm.	Probability ratio Inhibition
Saline control	970	
5γ *d*-desthiobiotin and 10γ "avidin concentrate"	1,011	0.6
5γ *d*-desthiobiotin and 20γ "avidin concentrate"	474	9.1
5γ *d*-desthiobiotin and 40γ "avidin concentrate"	517	8.0

The same relationship has been shown for the system leucopterin-xanthopterin (5). However, when leucopterin, a very potent antagonist for xanthopterin, was tested with inositol, an inhibitor of tumor growth, there was only slight interference. This fact suggests the possibility of a specificity in antagonism. Thus it may well be that the closer the chemical relationship, the more effective will be the neutralization or antagonism in that the antagonist acts at the same point at which the inhibitor interfered with the mechanism of tumor growth. However, both *p*-aminobenzoic acid and pyridoxin were able to counteract completely the tumor-growth inhibition produced by inositol; yet no chemical relationship exists between these substances. Thus there can be complete neutralization or, in terms of our experiments, a biochemical antagonism, even though no direct chemical relationship can be demonstrated.

The antagonism of two tumor growth inhibitors, desthiobiotin and avidin concentrate, creates a problem that is difficult to understand. A combination of desthiobiotin and avidin has been shown to occur *in vitro* by Melville and his associates (7), but in the experiments reported here the dose levels at which the interference occurred were such that the mere binding of desthiobiotin by the avidin concentrate does not supply a satisfactory explanation, since 10γ of avidin concentrate neutralized 5γ of desthiobiotin. It may be that the unknown impurities in the avidin concentrate exerted some effect.

Although in the studies reported here definite interference has been shown to occur between inhibitors of tumor growth and their antagonists, chemically related or unrelated, it is impossible at this time to state whether these results have any bearing on the chemotherapy of malignant tumors. Further investigations with various rapidly growing tissues, malignant and nonmalignant, should be carried out. As the substances tested to demonstrate this phenomenon of interference or neutralization were physiologically active, further studies are under way with simpler or non-physiological compounds.

SUMMARY

By adapting a method that detects inhibitors of tumor growth it is possible to demonstrate that the action of inhibitors can be effectively neutralized by both structurally related or unrelated substances.

Neutralization by approximately equal amounts of inhibitor and antagonist was observed in the inositol: *p*-amino-benzoic acid, inositol:pyridoxine, and *d*-desthiobiotin:*d*-biotin experiments. Thiamin, niacinamide, *o*- and *m*-aminobenzoic acid, and leucopterin were slightly active, if at all, in counteracting the inhibition caused by inositol. Interference could be detected when larger doses of some of these substances were given.

While both *d*-desthiobiotin and an avidin concentrate were effective inhibitors of tumor growth, neutralization occurred when these two materials were tested for antagonism. Impurities in the avidin concentrate may be responsible for this interference.

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Intraperitoneal Sarcomas Produced in Mice With Mouse Ascitic Fluid

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When a pellet made of 0.1 cc. of paraffin containing 1 mgm. of methylcholanthrene was introduced into the abdominal cavity of 62 young adult male C57 mice the abdomen became distended with fluid after 50 to 100 days. With the exception of those animals that died from intercurrent causes all developed intraperitoneal sarcomas. When 1 or more injections of ascitic fluid from these pellet-bearing animals was made into the abdomen of a new series of the same strain, ascites and sarcomas followed in a short time. The ascitic fluid from these mice elicited ascites and sarcomas in a third series.

When the abdomen became so tense that the mouse had difficulty in moving about the cage the fluid, usually 8 to 10 cc. in amount and clear or cloudy, colorless or ranging from straw color to deep red, was aspirated. It was centrifuged for 10 minutes at 1,200 r.p.m. and the supernatant layer poured off and passed through a Seitz filter. If the fluid was still viscous it was diluted with an equal amount of sterile saline solution to facilitate filtering. All procedures were carried out under aseptic conditions. Smears of the filtered fluid showed no cells. The fluid was examined also under filtered ultraviolet light but the characteristic methylcholanthrene fluorescence was not found. Furthermore fluorophotometer-galvanometer readings showed the absence of even infinitesimal traces of methylcholanthrene. On several occasions the fluid was cultured for 48 hours on thyoglycolate medium without obtaining any growth.

MATERIAL AND METHODS

Of 62 animals bearing the methylcholanthrene pellets 10 died from intercurrent disease before 50 days had elapsed. Beginning with 50 days after the pellets had been introduced the survivors began to show abdominal distention. Nine died with fluid in the abdomen before they had been tapped and most of these had abdominal adhesions, which in 2 animals caused intestinal obstruction. None had tumors.

Of the 43 remaining animals all that survived 90 days after placing of the pellets developed ascites and sarcomas.

Eighty young C57 black males were injected intra-abdominally with 0.5 cc. of ascitic fluid obtained as described above. One of these died 18 days afterward and 2 tumors, measuring 8×4 mm. and 11×5 mm. respectively, were found in the pelvic cavity. They proved to be mixed cell sarcomas (Fig. 1).

Of the 79 remaining animals 48 died of intercurrent disease, and 31 developed intra-abdominal tumors after the following periods:

41 days		102 days	
54		103	
55		104	
58		110	(4 animals)
65	(2 animals)	114	(2 ")
88	(2 ")	116	
90		119	
92		120	
93	(2 ")	123	
98		126	
101	(2 ")	149	
		162	

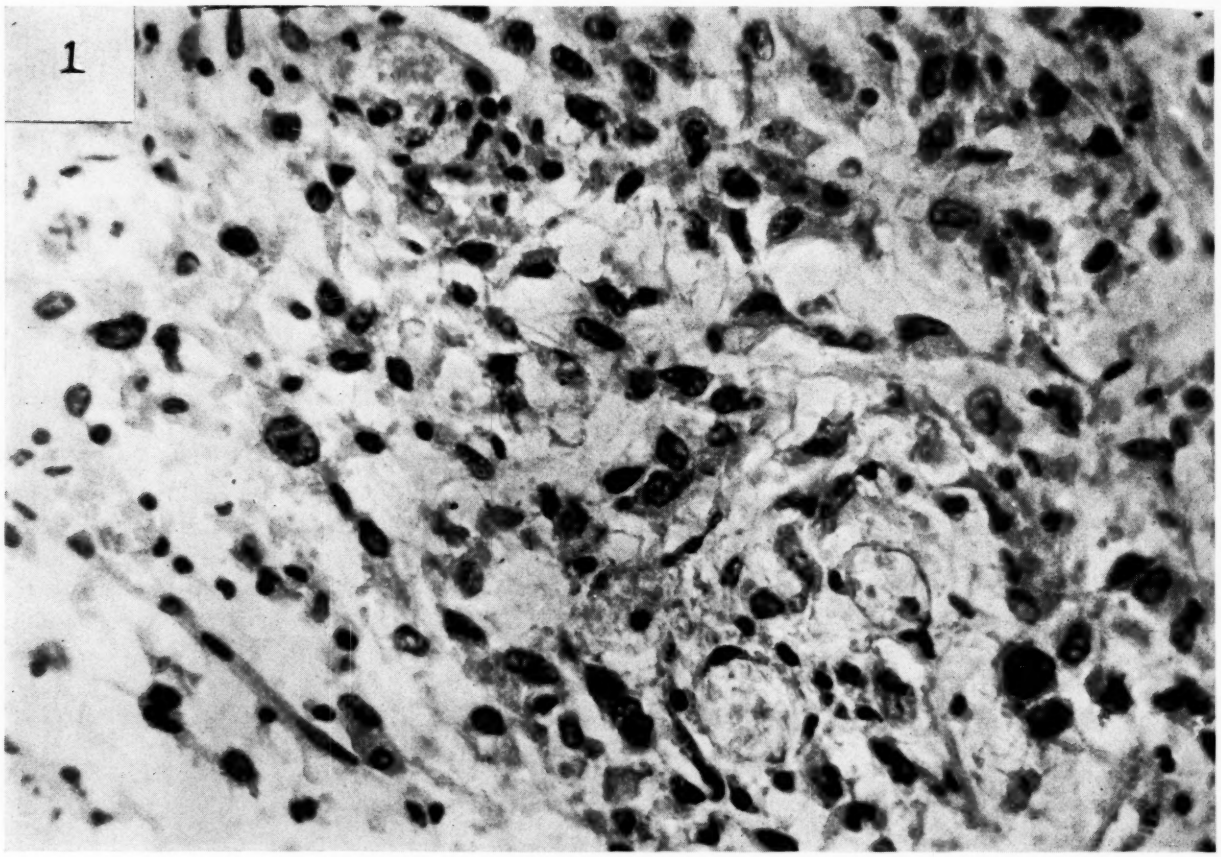
The ascitic fluid that collected in them was injected into a third series of 12 C57 black males, 7 of which developed tumors at 109, 116 (2 animals), 124, 126, 130, and 133 days.

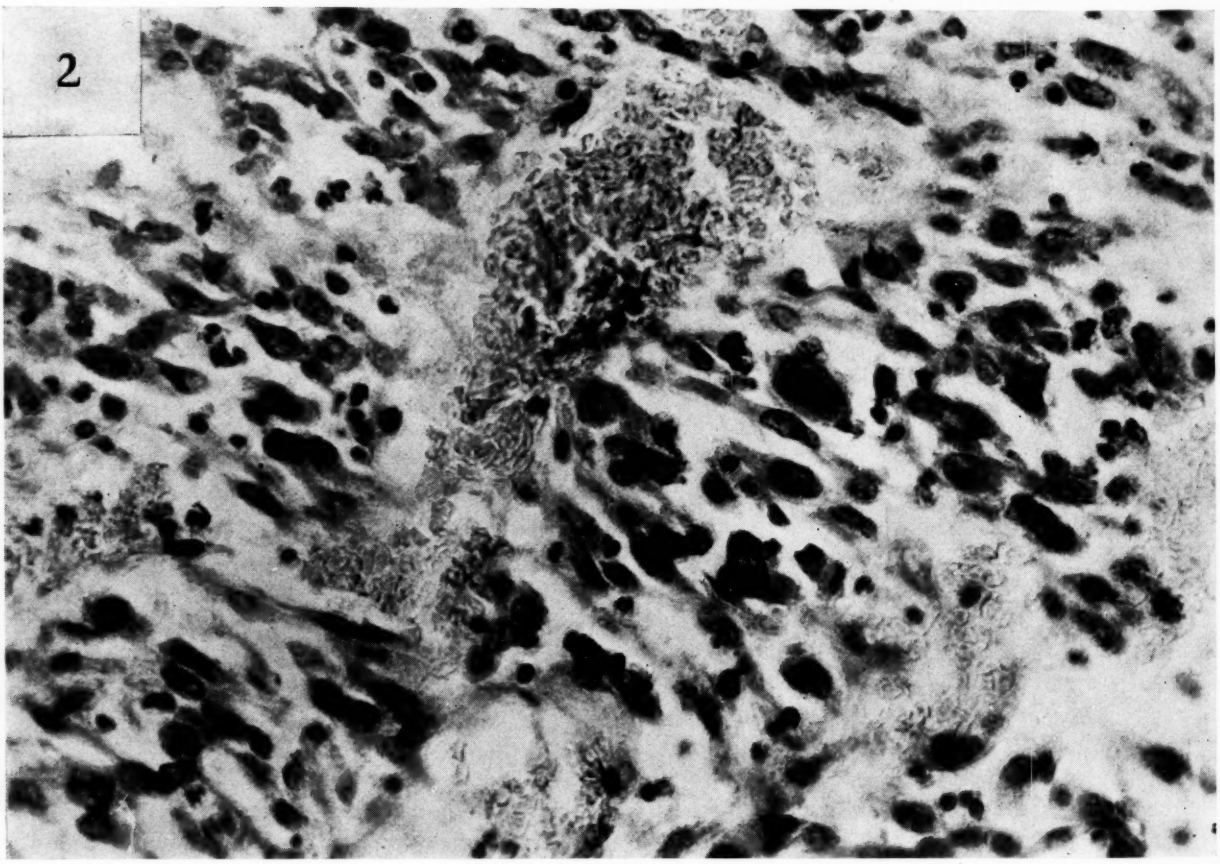
All the tumors found in animals injected with ascitic fluid were sarcomas, mostly of the mixed cell variety and many showing myxomatous features.

The abdomen of one mouse 65 days after injection with ascitic fluid contained many globular, partly translucent masses floating in the ascitic fluid, most of which were discrete; some of the larger ones were attached to the diaphragm. On microscopic examination these globules were found to consist of myxomatous cells; the larger globules were attached to organs, and showed features of malignancy with infiltration into the neighboring tissues (Fig. 2).

The subcutaneous transplantation of several of these tumors into 13 C57 black males resulted in 5 takes, all typical sarcomas.

In a discussion with Dr. R. Norman Jones on the nature of the agent responsible for these sarcomas he said that the destruction of activity by heat would not necessarily indicate the presence of a virus-like prin-





FIGS. 1 AND 2

ciple, since the tumors might have been elicited by a heat-labile metabolite of methylcholanthrene. He kindly prepared an ether extract, which was very small in amount. It was suspended in 3 cc. of sterile peanut oil and 0.5 cc. was injected intra-abdominally into 6 RIII male mice but neither ascitic fluid nor tumors developed.

The residue left after ether extraction was mixed with peanut oil, centrifuged, and sterilized by heat, and 0.5 cc. was injected intra-abdominally into 10 RIII male mice. Neither ascites nor tumors were obtained although in a control experiment methylcholanthrene pellets produced both in male mice of this strain.

Whole ascitic fluid from mice bearing pellets was heated to 90° C. for one hour and injected intraperitoneally, 0.5 cc. each into RIII male mice. Neither ascites nor tumors resulted.

In order to determine whether ascitic fluid contained sufficient methylcholanthrene or a metabolite of this compound to elicit sarcomas when injected *subcutaneously,* 12 RIII male mice were injected with 0.5 cc. of ascitic fluid from pellet-bearing mice. No growths were obtained.

It is to be noted that tumors were sometimes elicited with ascitic fluid from the abdomen of mice that on subsequent autopsy had no demonstrable tumors.

It does not seem likely that heating would destroy the activity of a carcinogenic compound responsible for the ascites and the tumors unless this were some labile metabolite. For whole ascitic fluid heated for one hour at 90° C., when injected intraperitoneally, produced neither ascites nor tumors.

SUMMARY

1. Paraffin pellets containing 1 mgm. of methylcholanthrene, when introduced into the abdominal cavity of young adult male C57 mice, produced ascites in 50 days and sarcomas in all mice surviving for 90 days.

2. The ascitic fluid, even when obtained before the development of sarcomas, produced ascites and malignant tumors when injected intra-abdominally into another series of the same strain.

3. The ascitic fluid obtained from this second series, when injected intra-abdominally into a third series of mice, again resulted in ascites and sarcomas.

4. These tumors were transplantable by subcutaneous inoculation.

5. Whatever the active agent may have been, it was destroyed by ether and by exposure to 90° C.

Melanosarcoma and Rhabdomyoma in Two Pine Snakes

(Pituophis Melanoleucus)

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So few reports of tumors in snakes appear in the literature that it is desirable to record any new cases. No exhaustive search of the literature was undertaken. In a brief survey Lucké (5) lists only two specimens, both described by Bland-Sutton in 1885; one was a fibroma of the stomach, the other a carcinoma of the ovary with widespread metastases. Both tumors occurred in pythons. Ratcliffe (7) reported an instance of carcinoma of the pancreas in a pine snake, *Pituophis sayi*. In 1941 Mergman (6) stated that in 2,200 dissections of snakes he had found no macroscopic tumors.

The present report deals with melanosarcomas and a rhabdomyoma observed in a male and a female pine snake, *Pituophis melanoleucus*. Both specimens arrived at the San Diego Zoo from Michigan in 1936; they fed well in captivity and grew satisfactorily. I am indebted to Mr. C. B. Perkins, curator of reptiles at the Zoo, for much of the historical data.

Case 1

History.—In May, 1939, an irregular dark swelling was noted on the tail of the female at the border of one of the dark markings. During the next 5 months this increased so in size that by October, 1939, the snake was no longer suitable for display (Fig. 1). The tail and attached tumor were amputated in February, 1940; the growth was diagnosed a malignant melanoma and the likelihood of future metastasis indicated. This opinion was substantiated by the subsequent appearance of 2 dark subcutaneous nodules about the head and 1 on the abdomen. All were removed by electrocautery.

Two months after the amputation the animal laid 6 eggs, all of which hatched. The young were entirely normal. At this time the snake weighed 1.73 kgm. and measured 157 cm. in length. In January, 1941, she again mated with the male to be described below, and laid another batch of 6 eggs. Three of these hatched, though one of the young was blind, the eyes being unusually small. Of the 3 young that failed to hatch out, 2 likewise had abnormal eyes.

In April, 1941, a large, deeply situated swelling was apparent near the tail. The snake ceased feeding properly at this time, and although she ate a medium sized rat in July she disgorged it 2 days later. Death occurred on October 12, 1941.

Autopsy.—A symmetrical fusiform enlargement of the body extended forward from the cloaca for a distance of 12 cm. The overlying skin was intact. On opening the abdominal wall in this region a firm, friable, ovoid mass $11 \times 6 \times 5$ cm. was seen filling most of the celomic cavity (Fig. 2). It compressed the intestine and prevented the passage of 2 large boluses of hair and bone, remnants of rats previously eaten. The tumor was encapsulated and did not invade the surrounding tissues except at its site of origin.

On each side of the tail, 3 to 4 cm. distal to the cloacal orifice, was a subcutaneous nodule. Both were well circumscribed, ovoid, gray-black in color, and similar in consistency and appearance to the intra-

DESCRIPTION OF FIGURES 1 TO 6

Fig. 1.—Gross appearance of primary tumor, Case 1, in October, 1939.

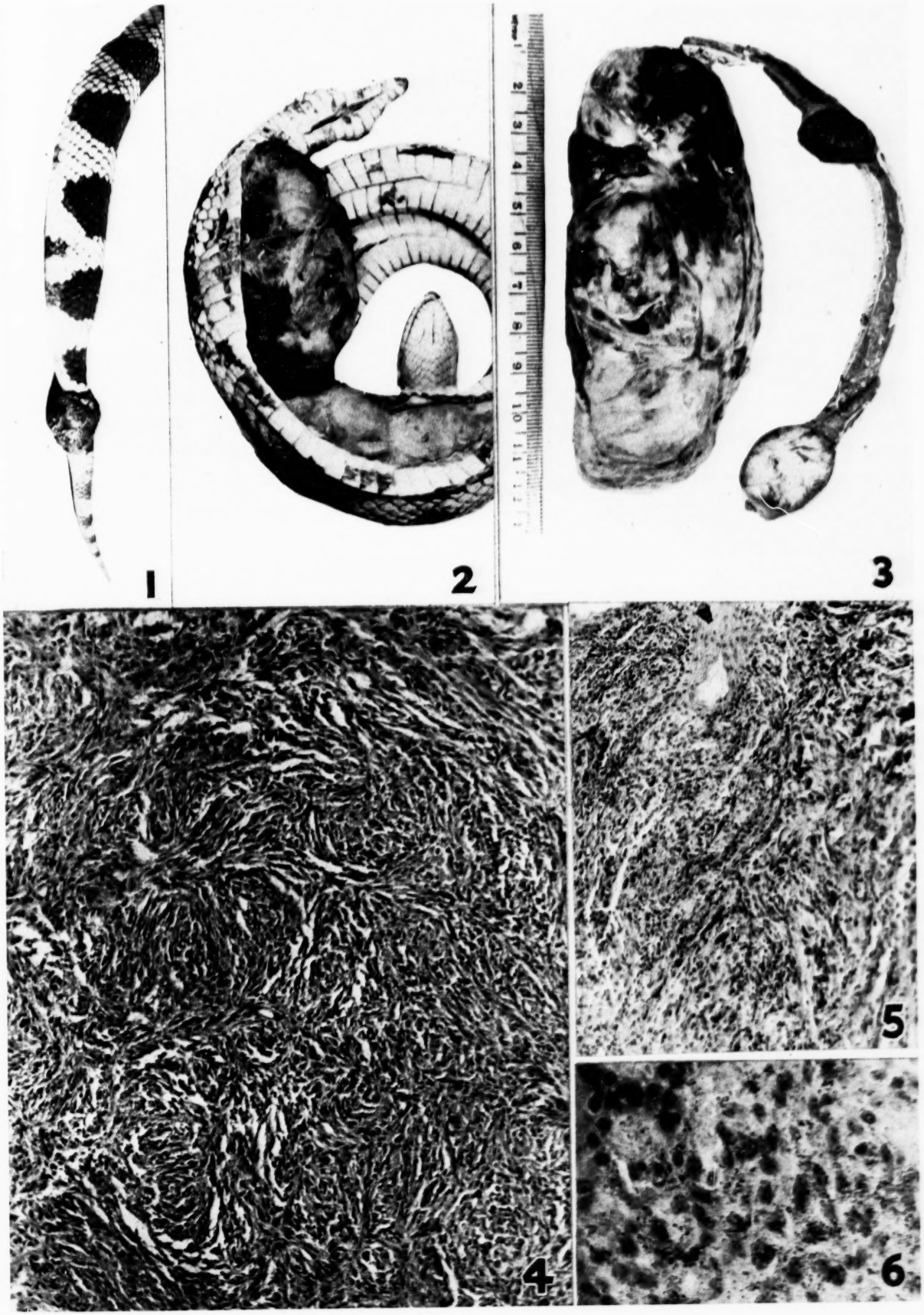
Fig. 2.—Gross appearance of metastatic tumor in celomic cavity of Case 1. Note also small subcutaneous tumors on each side of stump of tail.

Fig. 3.—Gross appearance of metastatic tumor removed from celomic cavity, and of 2 metastatic tumors in liver; one of the latter is richly melanotic, the other is but lightly pigmented. Case 1.

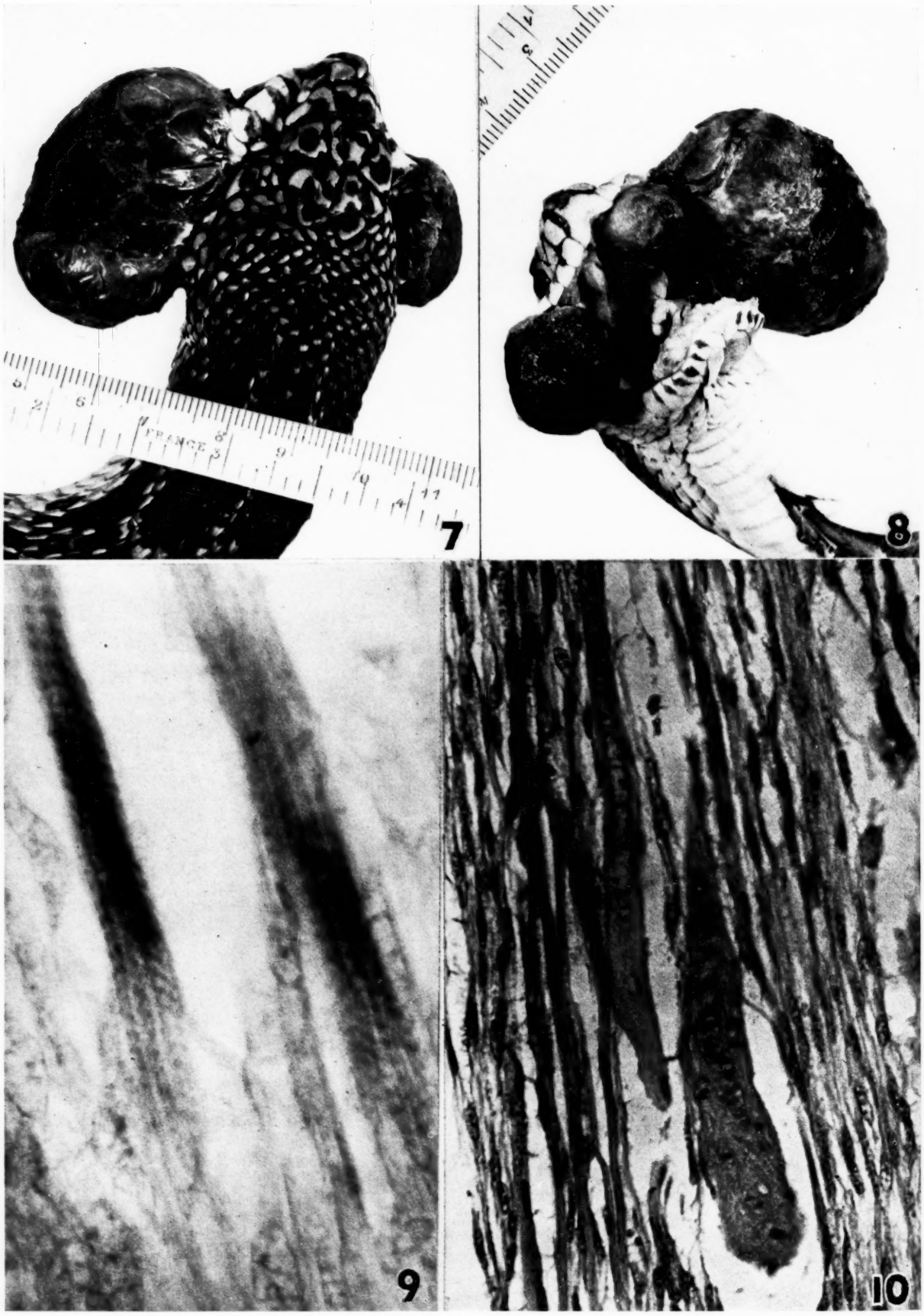
Fig. 4.—Tumors shown in Figs. 1 to 3 are composed of interlacing bundles of spindle-shaped cells. Mag. × 110.

Fig. 5.—This photomicrograph is representative of pigmented portions of tumor shown in preceding figure. Mag. × 100.

Fig. 6.—Pigment within tumor cells in form of small, brown granules. Mag. × 300.



FIGS. 1-6



FIGS. 7-10

abdominal tumor. One measured 1.3×3 cm., the other 1.1×1.5 cm. Examination of the viscera disclosed 2 spherical nodules in the liver; one was light gray in color and measured 2.5 cm. in diameter; the other, 2 cm. in width, was black throughout (Fig. 3).

Histology.—The tumor was made up of spindle-shaped cells arranged in bundles with a tendency to interlace (Fig. 4). The cells had a generous amount of cytoplasm, which was slightly acidophilic, fibrillated, and often contained a brown, finely granular material (Figs. 5, 6) that did not give the Prussian blue reaction and was assumed to be melanin. The nuclei were large and oval, and occasionally those of adjacent cells were bunched together, leaving irregular cytoplasmic fields without nuclei between them. This was reminiscent of the nuclear palisading displayed by schwannian syncytia. The metastatic lesions in the liver were histologically identical with the primary tumor.

Case 2

History.—A tumor was first noted on the left upper labial fold of the male snake on February 4, 1942. At that time it was estimated to be not more than 1 cm. in diameter. By August 25th it had increased considerably in size. The animal was sacrificed in November, 1942.

Autopsy.—The snake was 170 cm. long, appeared fairly well nourished, and was free of gross abnormalities except for the lesions about the mouth (Figs. 7, 8). Arising from the entire posterior half of the left upper labial fold was a 4×2.8×2 cm., ovoid, dark gray tumor; it was covered by thin, greatly expanded scales and bore a central, irregular, shallow area of ulceration. A similar lesion, measuring 2.5×1.5×1.5 cm., was present in the identical position on the right upper labium. A 1.5 cm., spherical, nonpigmented, and broadly sessile mass occupied the left anterior portion of the palate. Inspection of the viscera, including multiple sections through the liver, failed to disclose any metastatic lesions.

Histology.—The morphology of the pigmented labial tumors was the same as that of the lesions in the preceding case. However, the histology of the tumor of the palate was wholly different from that of the adjacent pigmented ones. Greatly elongated cells arranged in large bundles predominated. The large, nearly rectangular nuclei were grouped about a fibrillated syncytium, and in many instances distinct cross striations were visible (Fig. 9). Scattered through the section were plump giant cells with centrally located, multiple nuclei (Fig. 10). The cytoplasm of these cells was likewise frequently striated. The histological picture was typical of a rhabdomyoma.

DISCUSSION

The occurrence of malignant melanomas in reptiles is of considerable theoretical interest with regard to the recent work of Laidlaw and Murray (4) on human pigmented moles. These investigators believe that the latter is a phylogenetic tumor that represents an abortive tactile spot related to the well-developed structures normally found in reptiles. The arrangement of the spindle cells in the snake lesions was reminiscent of a schwannian cell tumor, and silver stains showed occasional neurites in the stroma; nevertheless, it cannot be stated with certainty that they were derivatives of the neuroectoderm rather than of the epithelial melanoblasts. No intimate relation of the tumor with the tactile spots could be demonstrated.

The location of the melanoma was comparable to that in man. In a review of human melanosarcomas of the oral mucosa, Fuhs and Kumer (2) recorded 29 cases and added 2 of their own. The gingiva was second only to the hard palate as the most common site of origin. In only 1 instance did the lesion arise in the lower gum; in the remainder only the upper was involved. With this in mind it is interesting to note that both gingival tumors of Case 2 were on the inner aspect of the upper labial folds.

A possible genetic effect of the disturbed pigment metabolism in the parents is indicated by the malformed eyes of the offspring. That this is not wholly improbable is indicated by the experiments of Hale (3), who found that the pigs farrowed by a sow kept on a vitamin A deficient diet were completely devoid of eyeballs. Since vitamin A is closely related chemically to the lipochrome constituent of visual purple, this experiment is of interest as an example of impaired pigment formation affecting normal development of the eyes.

Although rhabdomyomas are known to occur in the tongue of man, striated muscle tissue in tumors of the palate has usually been associated with teratomas. In a monograph on axial bifurcation in serpents,

DESCRIPTION OF FIGURES 7 TO 10

Fig. 7.—Gross appearance of tumors of Case 2, as seen from above.

Fig. 8.—Gross appearance of tumors of Case 2, as seen from below. Two lateral tumors are melanomas, middle tumor is a typical rhabdomyoma.

Fig. 9.—Middle tumor, shown in preceding photograph, composed of large, elongated cells, many of which have prominent cross striations. Mag. × 250.

Fig. 10.—Two multinucleated muscle giant cells are shown in central part of photomicrograph. Mag. × 435.

Cunningham (1) emphasized the relative frequency of anterior duplication (dicephaly) in snakes. Lesser developmental faults about the head may also be quite common; hence the rhabdomyoma of Case 2 might represent a teratoma in which striated muscle had replaced the other tissues.

SUMMARY

Malignant melanomas occurring in a male and female pine snake are reported. The primary tumor in the female snake arose at the margin of one of the large pigmented areas of the skin of the tail. Metastatic tumors were found in the liver and the celomic cavity. In the male snake 2 large melanomas occurred on the upper lip, and another tumor, a typical rhabdomyoma, sprang from the hard palate. These growths appear to be the third or fourth instances on record of malignant neoplasms in snakes.

ACKNOWLEDGMENT

The author wishes to express appreciation to Lt. Col. Balduin Lucké for his kindness in studying these tumors, for the suggestions made, and for assistance in the preparation of illustrations.

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Betel Chewing Among Natives of the Southwest Pacific Islands

Lack of Carcinogenic Action

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In general discussions on the relationship of natural compounds or agents to the development of neoplasia, it is frequently suggested that betel chewing is a factor in the etiology of oral cancers throughout those portions of the world where betel mixtures are in use. To some the habit would assume an importance comparable with roentgen or solar radiation, or tar, in the production of tumors of the skin. A specific incriminating factor or substance in the materials employed by betel chewers has not been described, neither is there unanimity of opinion regarding the activity of the betel mixture. Betel chewing is an almost universal indulgence in many regions of southeastern Asia, in the small and large islands adjacent to the coast, and in the Southwest Pacific area.

The reports of Davis (2) and of Mendelson and Ellis (5) would indicate that a relationship exists between betel chewing and the higher incidence of oral cancer in districts of the Philippines and Siam respectively. Davis says the active agent is the lime of the chew. The experiences of Orr (6) and of Friedell and Rosenthal (3), however, appear to negate the carcinogenic potency of the habit. The former found that oral cancer is uncommon among Hindus of the Bilhar district, who chew betel incessantly, but does occur in an aboriginal tribe in the same district who chew tobacco and lime, but are not users of betel. The latter authors likewise stress this possible relationship of irritating tobaccos to oral neoplasms. In an attempt to evaluate experimentally some of the components of the betel chew, Woelfel, Spies, and Cline (9) tested the ether and alcohol extracts and the unsaponifiable fraction of the betel (areca) nut on mice, but failed to elicit tumors.

The purpose of the present communication is to relate the experiences of Australian workers and the present author among the natives of New Guinea. The latter, as a member of a United States Army General Hospital, has had access to the observations of Australian medical personnel directly responsible for the health of the inhabitants of this and other islands of the Southwest Pacific area, and is grateful for the data furnished him and the facilities for examining and interviewing a considerable number of natives during a period of 14 months. By far the major portion of this time has been spent in Northeast New Guinea. The length of stay in Papua was much shorter. In reporting these experiences and comparing them with the findings of other observers from other sections of the world, it is realized that variations in local terminology exist where the betel habit is practiced; that there are differences in the content of the chew; and, finally, that accurate statistical evaluation is out of the question among peoples who frequently have difficulty in establishing their correct age or who, in some instances, live in relatively remote localities that are rarely visited by competent, trained medical personnel.

As in portions of Asia and many Pacific islands, betel chewing is universally practiced among the natives of New Guinea, New Britain, New Ireland, and the neighboring smaller islands that have been under Australian control. It is begun in adolescence in both sexes, although a somewhat larger percentage of females are nonchewers. It is the practice of the Australian authorities to discourage the habit in men employed in the native constabulary or as attendants in institutions devoted to the care of the indigenous sick. The reason offered is its possible interference with duties. The natives are in general a cooperative and intelligent group, and those in contact with the whites speak a pidgin dialect that can be understood or interpreted without great difficulty. They are not reluctant to answer questions regarding their customs and beliefs. Williams (8), in an excellent ethnographic study of the Orokaivas, a tribe of Papua, states that betel chewing exerts a mild, comparatively harmless, stimulating action, making life more pleasant for the users. In some ways it is their equivalent of the alcoholic beverages of other peoples, for use of the customary intoxicants appears to be unknown to the natives of New Guinea. While differences exist in the physical characteristics, dialect, and tribal cus-

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toms of the various native groups, they are not sufficiently great to invalidate generalizations on their habits and reactions to environmental factors. However, it is quite probable that those natives who are employed by the Australians, or have local agricultural products for trade, receive a better balanced diet as a result of added amounts of meat and unpolished rice obtained from the whites. The importance of these dietary variations is difficult to establish.

The Betel Chew

The preparation of the betel cud is fairly uniform in this part of the world, with variations only in the source of the lime, an important component. The other constituents are the areca nut (*Areca catechu*), commonly called the betel nut, and the leaves or pods of the piper betel. The areca nut varies from 3 to 5 cm. in length, occurs plentifully on tall, slender palms, and is composed of an outer tough, yellow-green husk and an inner pulp that is pale yellow or slightly pink-tinged in the more mature variety. This is referred to locally as *buc*. The piper plant, called *daka* by the natives, is a vine-like structure, and the leaves or the long, thin pods are employed. The preference appears to be for the pods, but as the plant contains a relatively smaller number of them the leaf, obtainable in large quantities, is more commonly used. Some variation in the preparation of the lime, the final substance of the mixture, occurs; on New Guinea it is generally obtained from sea shells, which are slowly dried over a fire and then reduced to a white powder of remarkably fine consistency. On the islands of New Britain, New Ireland, and the adjacent smaller groups, where coral is more easily obtainable off-shore or immediately below the surface soil, the lime is prepared from it by a similar process of desiccation and pulverization. Lime, in native dialect, is referred to as *kumbung*.

Chewing is practiced by first inserting a piece of the areca nut pulp in the mouth. This is masticated for a few seconds, following which the piper leaf or pod, with a generous helping of lime, is taken; a large wad results, which rapidly assumes a brilliant red color and, with long-continued indulgence the tissues of the oral cavity become stained bright red. The mixture induces intense salivation. The nut is highly astringent, but this unpleasant effect is neutralized by the lime. As can be demonstrated in the test tube, the lime is alkaline, and its use has without doubt been evolved by the natives through trial and error, for it is universally available in unlimited quantity, prepared simply, and they know from experience the amount required to eliminate the irritating effect of the nut alone. The value of the piper plant is difficult to establish, although it is not beyond possibility that some of the influence of the chew may result from active principles that it contains. The natives insist that the betel mixture is unsatisfying without it, and it is certainly true that the characteristic red color of the wad will not appear without all three ingredients. There is the question, too, of the psychologic influence of the red color. Systemic effects are produced by the mixture: exhilaration, sleeplessness, and, on overindulgence, ocular disturbances. The natives chew daily, although they observe no fixed schedule. The wad is expectorated when the chewer feels that he has obtained the maximum benefit from the mixture.

Use of Tobacco

Tobacco has been a favorite luxury with the peoples of New Guinea and other islands ever since it was introduced by white settlers. Smoking is not limited to either sex, although the percentage of males who smoke is higher, and the habit is followed much as in other countries, though with several exceptions. It is frequently begun in the preadolescent years. The popular tobacco is a cheap, coarse American twist, a favorite trade object, and it is employed either in long cigarettes, for which the natives like newspaper as a wrapper, or in pipes. A native tobacco, called *brus,* is also smoked in the same fashion, after preparation by simple drying in the sun. Although the natives are not averse to smoking while chewing betel, they do not chew tobacco.

The question of the relationship between tobacco and oral tumors has been raised by some investigators, but no proof one way or the other can be established from observations on the New Guinea natives, as will be demonstrated in the section of this report describing tumors observed here.

Some Properties of the Substances of the Betel Chew

The areca nut has an acid reaction. The lime is highly alkaline, and consists principally of calcium carbonate, with calcium oxide as the agent responsible for the alkalinity. The color change of the nut, piper, and lime occurs *in vitro,* saliva being unnecessary. The color appears after 20 to 30 seconds in the fibrous septa subdividing the nut into compartments. An aqueous solution of the piper leaf or pod will effect the same color change, which can be proved to be dependent upon the alkaline reaction, not the calcium, for a combination of nut, piper, and sufficient NaOH to produce alkalinity will result in the same alteration as occurs with native lime. This change will not occur upon the substitution of pure calcium carbonate for the alkalinizing substance.

Clinical Observations

These have been furnished largely through the cooperation of the Australian Army Medical Corps, in whose hands rests the immediate responsibility for the health of the natives, and though the data are necessarily only approximate, this does not detract from the general value of the records. Captain J. R. Waddell (7), for example in a series of 8,000 natives admitted to the Angau Hospital, in Northeast New Guinea, observed only 1 case of squamous cell cancer of the oral mucosa; this was in a male aged 35. The age of the patients in this institution varied from 16 to 40 years, with the larger number falling within the range of 25 to 35 years. Of the total number, 90 per cent were from a workers' compound, the population of which is 90 per cent male. The nutritional status of these patients is superior to that of the inhabitants of native villages near by, who comprise 10 per cent of the admissions. During several years' patrolling, as the periodic visits to native settlements for health survey are called, Waddell failed to record a single example of oral cancer among the inhabitants of New Ireland and small adjacent islands. The natives there pursue lives substantially identical with those on the New Guinea mainland.

Captain H. I. Jones (4), Australian Army Medical Corps, saw 4,000 admissions to the native Angau Hospital, in Papua, of an age group comparable with that of Waddell, and observed only 1 case of squamous cell epithelioma of the tongue in a young female.

The experiences of Major T. C. Backhouse (1), Australian Army Medical Corps, are valuable. Working in the Government Health Laboratory on New Britain between 1921 and 1940, this investigator collected 60 examples of malignant neoplasm among the natives, the majority of which were of the types usually observed in any population, with a single exception: primary cancer of the liver, with associated cirrhosis, was one of the common neoplasms. There were 2 carcinomas of the lip; 4 of the oral mucosa, of which 2 extended through the cheek; 1 undifferentiated carcinoma infiltrating the hard palate; and 2 primary cancers of the stomach with regional and hepatic metastases. Histologic preparations of selected examples of this group of tumors were examined by the present author.

Major W. D. Wolfe (10), Medical Corps, A. U. S., analyzed the incidence of lesions of the skin and mouth in 1,047 natives of New Guinea, predominantly males of the age groups observed by Waddell in the Angau Hospital. While fungus diseases and scabies were found to be common, mouth lesions proved unusual. Not a single instance of leukoplakia was encountered. This is in harmony with the general experience of Australian investigators, who likewise have noted the infrequent incidence of alterations in the mouth that might be deemed precursors of malignant disease. The teeth of the betel chewers, although stained red, are in good condition. With time they become darkened, concomitantly with the deposition of calcareous material.

The general impression, therefore, is that no unusual incidence of oral lesions occurred among this group of natives. As the betel-chewing habit is so widely practiced among the peoples in question, it is obviously impossible to determine the frequency of tumors of the mouth in a comparable series of nonchewing controls.

SUMMARY

Betel chewing in the natives of New Guinea, New Britain, New Ireland, and the adjacent smaller islands does not appear to elicit cancer of the mouth.

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Histologic Changes in the Central Vegetative Centers of the Hypothalamus in Carcinoma as an Indication of Vegetative Functional Disturbances

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Alone and in collaboration with others I have made a histological study of the hypothalamus in approximately 200 human brains, from patients exhibiting a wide variety of clinical conditions.

A review of the pathologic changes occurring in chronic and acute psychoses, epilepsy, and mental deficiency, with a discussion of the possible meaning of these changes, was published in a recent paper (7); the changes of the hypothalamus in diabetes mellitus (8) and heat stroke (9) also have been recorded.

Our present knowledge—still far from complete—of the fiber connections and functions of the hypothalamus have been reviewed by Fulton (3), Masserman (6), Ingram (4), Ranson and Magoun (10), and others. Some of the functions that have been found to be influenced by the hypothalamus are temperature control; sleep; carbohydrate, fat, water, protein, and oxygen metabolism; and those of many viscera such as the cardiovascular, urinary, and digestive systems. There is much evidence to support the view that the nuclei of the hypothalamus influence the autonomic nervous system and the endocrine glands. Beattie, Brow, and Long (2), Fulton (3), and others have advanced the view that the anterior hypothalamus exerts its influence upon the parasympathetic, while Bard (1), Rioch and Brenner (11), and others conclude that the posterior hypothalamus is concerned with the excitation and integration of sympathetic reactions.

The evidence available indicates that the cell groups of the hypothalamus cannot be considered as separate centers, each having a single specific function comparable to the lower centers distributed throughout the brain stem and spinal cord. In the hypothalamus many vegetative functions are brought under a central mechanism, coordinated and controlled for a broader purpose and in the interests of the total body economy.

The anterior and posterior hypothalamus each give rise to a fiber tract that descends to the mid-brain, medulla, and spinal cord. The supraoptic nucleus sends most of its fibers to the hypophysis, which receives some fibers from the posterior hypothalamus and from the paraventricular nucleus of the anterior hypothalamus also. The anterior hypothalamus exerts its influence chiefly upon the parasympathetic nervous system and the islands of Langerhans, the posterior hypothalamus chiefly through the sympathetic nervous system and the thyroid and suprarenal glands, while the hypophysis is under the influence of both regions. It is apparent, then, that the hypothalamus utilizes to a large extent the autonomic and endocrine systems in achieving its broad purpose of coordinating vegetative functions in the interest of the total organism.

Thus it seems likely that the nuclei of the hypothalamus—and, perhaps, other closely associated centers outside the anatomical limits of the hypothalamus—are normally in a state of physiological balance. If this be true then disease, injury, or dysfunction involving one or more of the nuclei would probably tend to throw the entire mechanism out of balance, so that the effects would be more far reaching than might be expected were the nucleus concerned with a single specific function.

It is obvious that hypothalamic dysfunction will exist whenever disease or injury attacks the region directly. It is also possible that disease, injury, or dysfunction in any part of the body, that tends to throw vegetative functions out of balance or to threaten the welfare of the total organism, may eventually involve the hypothalamus because the central mechanism will be called upon to attempt a restoration of normal function.

Because of these possibilities the striking histological abnormalities that occur in the hypothalamus of the cancer patient may be of considerable significance.

HISTOLOGY

According to Malone (5), who classifies the nuclei of the hypothalamus on the basis of cell type rather than topography, there are 5 distinct cell groups.

The nucleus tubero-mamillaris.—This is composed of large cells, slightly smaller than those of the supraopticus, which they somewhat resemble.

They begin at the optic chiasm and extend past the oral half of the mammillary body, tending to group themselves around the medial, ventral, and lateral sides of the fornix. The cells increase in number toward the posterior end of the nucleus, and are most numerous at the oral end of the mammillary body (Fig. 1, A).

The nucleus tuberis lateralis.—The cells of this nucleus are considerably smaller than those of the other nuclei, with the exception of the substantia grisea. They have a relatively small nucleus with a large amount of cytoplasm, in which are seen minute, lightly staining Nissl bodies.

This nucleus consists of several groups of cells, more or less closely associated, embedded in the lateral or basilar portion of the tuber cinereum (Fig. 1, A). The more expanded part of the nucleus lies in the area ventromedial to the cerebral peduncle. These groups are so definitely circumscribed and so characteristic in appearance in the human brain that they are readily identified.

The substantia grisea.—The cells of this nucleus are smaller than those of any other cell group. There is considerable variation in their size, the smaller cells predominating close to the wall of the third ventricle. The cells possess relatively large nuclei, with a small amount of cytoplasm containing fairly large Nissl bodies.

This cell mass lies immediately adjacent to the third ventricle, extends through the entire length of the hypothalamus (Fig. 1, A), and is broadest posterior to the optic chiasm, where the cells spread laterally into the tuber cinereum and ventrally into the infundibulum.

The nucleus supraopticus.—This is composed of large, polygonal cells with very coarse processes. The Nissl substance is collected in large masses that are concentrated at the periphery of the cell, leaving a clearer area surrounding the nucleus.

This nucleus surrounds the beginning of the optic tract (Fig. 1, B). A larger cell mass lying anterolateral, and a smaller mass lying posteromedial to the tract, are usually connected by a thin layer of cells.

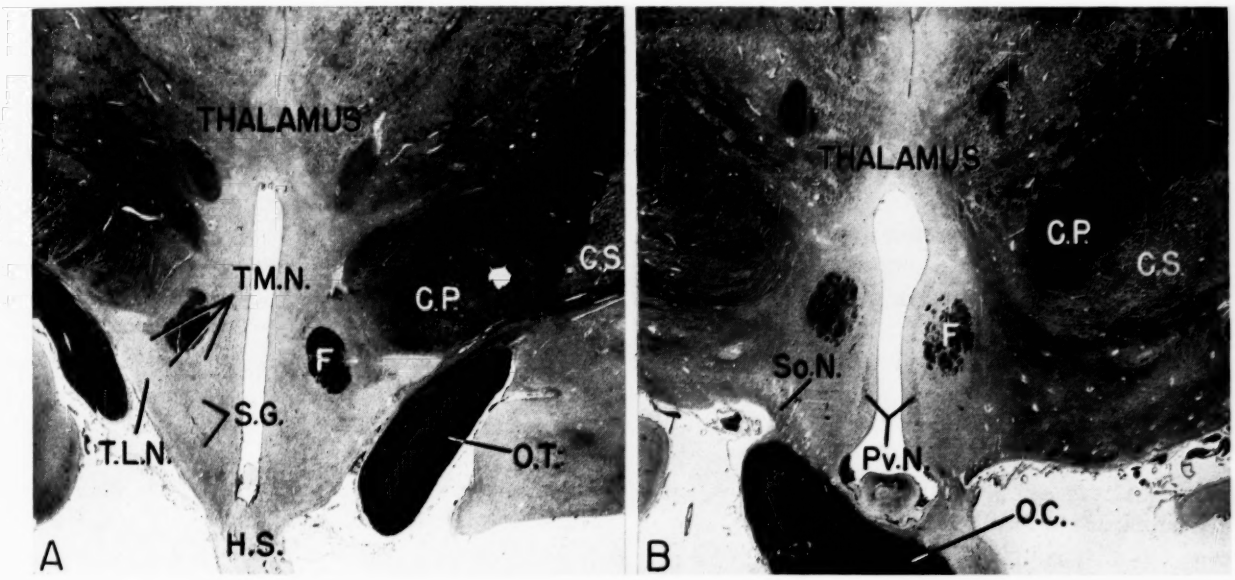

FIG. 1.—Sections through the diencephalon showing location of nuclei of hypothalamus. A: between the optic chiasm and the mammillary bodies. B: at the anterior level of the optic chiasm. C.S. indicates corpus striatum; C.P., cerebral peduncle; F., fornix; O.T., optic tract; O.C., optic chiasm; H.S., stalk of hypophysis; T.M.N., nucleus tubero-mamillaris; T.L.N., nucleus tuberis lateralis; S.G., substantia grisea; So.N., nucleus supraopticus; Pv.N., nucleus paraventricularis.

The nucleus paraventricularis.—The cells of this nucleus are similar in appearance to those of the supraopticus, but vary considerably in size.

The cells are arranged in an elongated column with its long axis perpendicular to the base of the brain, and its lower end situated just in front of the supraoptic nucleus (Fig. 1, B).

MATERIAL AND METHODS

This study is based upon 19 brains of cancer patients who came to autopsy from the Cincinnati General and the Hamilton County Chronic Disease Hospitals. After fixation in formalin the diencephalon was embedded in celloidin, sectioned 35 microns in thickness, and stained by the modified iron-hematoxylin method described in previous reports.

Cell loss in the hypothalamic nuclei was evaluated by counting the cells in at least two microscopic fields within each nucleus, and comparing the number with that obtained in corresponding areas of 10 control brains that were apparently normal. The percentage of normal and chromatolytic cells was recorded in each instance.

Abstracts of Clinical Cases

Case 1.—W. M., colored, male, aged 52, with a strongly positive Wassermann. Postmortem examination disclosed a carcinoma of the lung with metastases to the right fronto-parietal lobe of the brain, liver, suprarenals, ribs, and epicardium. There was cystic degeneration of the pituitary.

Case 2.—S. J., white, male, aged 67. On postmortem examination there were noted multiple pulmonary abscesses and adenocarcinoma (suggestive of prostatic origin); adenomatous hyperplasia of the prostate; cerebral neoplasm (glioblastoma multiforme) surrounding an area of softening in the right frontal and parietal lobes.

Case 3.—F. S., colored, female, aged 62. Postmortem examination revealed a carcinoma at the junction of the pharynx and esophagus.

Case 4.—M. S., white, female, aged 38. Postmortem examination revealed a hypernephroma on the right side with extension to the suprarenal, and with metastases to the hilar lymph nodes, lungs, and liver.

Case 5.—W. B., colored, male, aged 65. Postmortem examination revealed a lymphosarcoma with metastases to the kidneys and extensive invasion of the left ureter and the left common iliac vein.

Case 6.—J. R., white, male, aged 78. Postmortem examination disclosed an adenocarcinoma of the stomach with necrosis; adenoma of the pituitary with degeneration; leiomyoma of the colon; lymphangioma of the intestine; extensive encephalomalacia.

Case 7.—S. E., white, male, aged 64. Postmortem examination showed adenocarcinoma of the pancreas with metastases to the pleura, lungs, and liver; an old area of softening in the right caudate nucleus; generalized arteriosclerosis; and adenomatous hyperplasia of the prostate.

Case 8.—F. C., white, male, aged 48. Postmortem examination disclosed adenocarcinoma of the peritoneum, mesentery, and appendix, with acute fibrous peritonitis; recent active apical and hilar node tuberculosis; and low grade hepatic cholangitis with degeneration of the bile ducts.

Case 9.—A. W., white, male, aged 59. Postmortem examination showed an advanced adenocarcinoma of the stomach with metastases to the regional lymph nodes and the subcutaneous tissue near the umbilicus.

Case 10.—W. S., white, male, aged 57. At postmortem examination there were found an extensive gastric carcinoma with perforation and peritonitis; metastases to regional nodes, retroperitoneal nodes, pancreas, spleen, and lungs; and a meningioma involving the dorsolateral surface of the precentral, superior, and middle frontal convolutions of the right side.

Case 11.—E. B., white, female, aged 69. Postmortem examination revealed a carcinoma of the stomach and adenomatous hyperplasia of the thyroid.

Case 12.—M. Q., white, female, aged 74. Postmortem examination showed an epidermal carcinoma in the vagina and cervix with infiltration of the parametrium and a pararectal abscess.

Case 13.—R. C., white, female, aged 61. Postmortem examination disclosed metastatic adenocarcinoma in the brain (scattered throughout the cortex and striatum), lungs, liver, spleen, suprarenals, and iliac lymph nodes—probably primary in the fundus of the uterus; papillary adenoma of the uterus; syphilitic aortitis; tertiary syphilitic cutaneous ulcerations of the leg; syphilitic cirrhosis of the liver.

Case 14.—E. K., white, female, aged 58. At the postmortem examination there were found a malignant tumor of the anterior mediastinum involving all the anterior mediastinal structures, with partial occlusion of the superior vena cava; undifferentiated carcinoma of the thyroid.

Case 15.—W. J., colored, male, aged 48. The postmortem examination revealed a scirrhous adenocarcinoma of the stomach with metastases to the mesenteric, pancreatic, perigastric, periaortic, superior mediastinal, and supraclavicular lymph nodes.

Case 16.—M. C., white, female, aged 83. Postmortem examination showed a highly malignant anaplastic adenocarcinoma involving the pancreas, stomach, transverse colon, small intestine, kidneys, adrenals, ovary, mesentery, lung, and periaortic and periportal lymph nodes.

Case 17.—F. M., colored, male, aged 45. Postmortem examination disclosed a carcinoma of the pancreas, with metastases to the liver and the mesenteric and prevertebral lymph nodes.

Case 18.—L. M., white, male, aged 63. The postmortem examination showed a scirrhous carcinoma of the right side of the pharynx, with metastases to the surrounding lymph nodes, right pleura, right lung, and liver.

Case 19.—S. W., colored, male, aged 59. Postmortem examination revealed an adenocarcinoma of the prostate; mural thrombosis and myocardial degeneration; left hydrothorax; massive pulmonary and cerebral edema; diffuse cortical atrophy; focal cerebral softening and cortical degeneration.

Discussion of Findings

The study represents a miscellaneous collection of carcinomas of varied origin; some with extensive metastases, others with few or none.

In cases 1 and 13 there was metastasis to the brain, and in case 2 there was a glioblastoma. The involvement of the brain may have contributed to the cell changes in the hypothalamus. Case 5 was included because of a previous history of carcinoma of the prostate, although the terminal neoplasm was a lymphosarcoma.

The histologic alterations in the hypothalamus are illustrated in Fig. 2, where the cases are arbitrarily arranged in order according to the cell count in the nucleus tubero-mamillaris. This was done in order to show graphically to what extent, if any, the cell loss and chromatolysis in the various nuclei conformed to a uniform pattern. One hundred per cent represents the average cell count found in the group of control brains.

The graph shows a striking variability of both the chromatolysis and cell count as we compare the degree of involvement of the five nuclei concerned. There is a slight tendency for the curve representing the cell count to ascend for the supraoptic and paraventricular nuclei as it does for the tubero-mamillaris. However, the exceptions to this rule are more numerous than the cases that conform. We can conclude only that the pattern of involvement of the nuclei of the hypothalamus in carcinoma is rather variable.

There is a striking number of instances in which the cell count is considerably above normal. This is true particularly of the nucleus tubero-mamillaris, and to a lesser degree of the nuclei supraopticus, tuberis lateralis, and paraventricularis. In 12 cases the cell count for the nucleus tubero-mamillaris ranged from

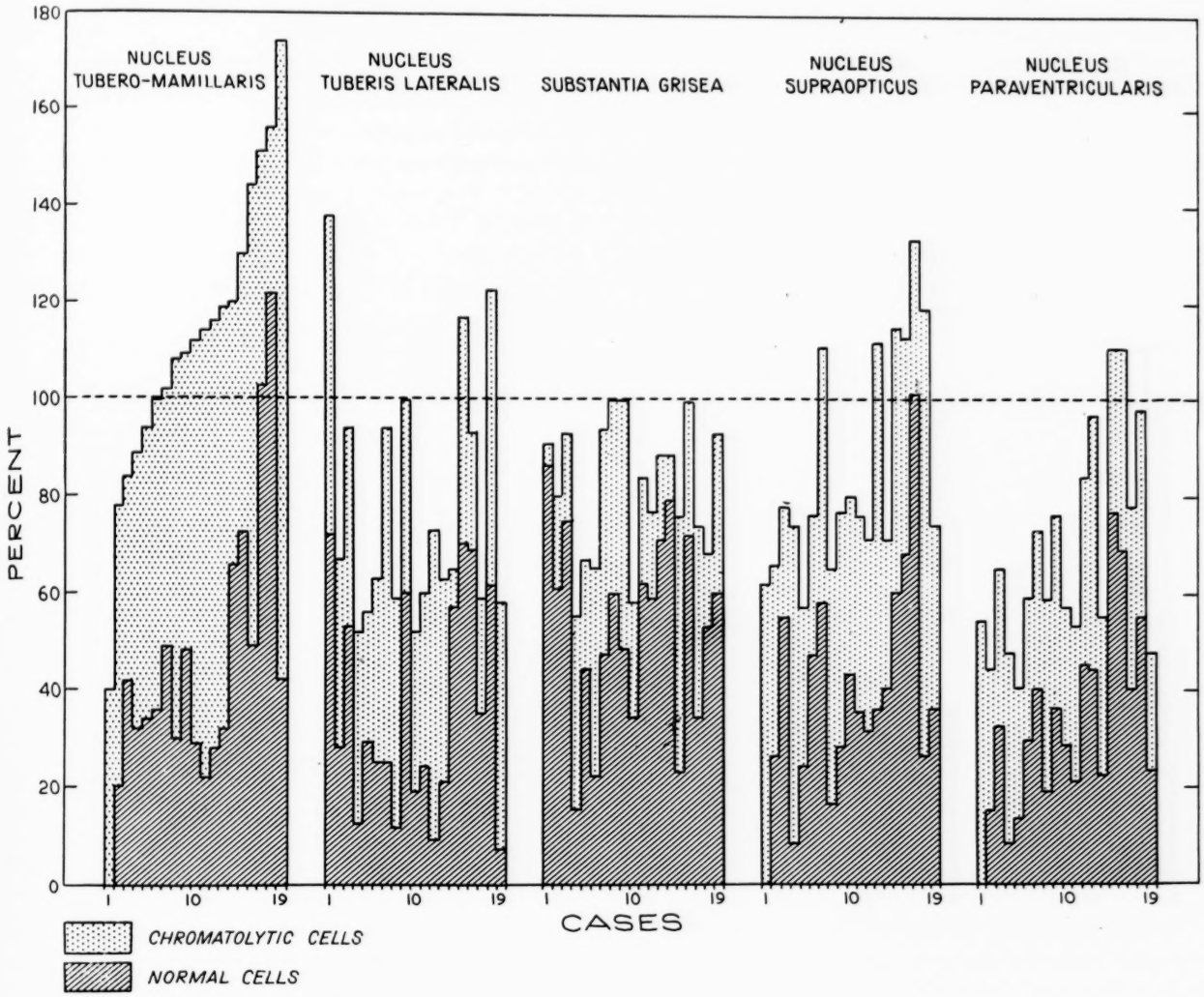

Fig. 2.—Cell count and percentage of chromatolytic cells in hypothalamic nuclei in 19 cases of carcinoma. 100 per cent represents average cell count in a series of 10 control brains.

8 to 74 per cent above normal. The average cell count for this group was 29.4 per cent above normal. To check the significance of this deviation a second group of 15 brains, showing no obvious abnormalities in the hypothalamus, was studied. This second group showed an average cell count of 103 per cent, as compared with the original 10 control cases.

The only condition noted in the hypothalamus of cancer patients that would suggest a congenital anomaly was this tendency toward overdevelopment in some of the nuclei. Considering the widespread cell destruction that occurs in carcinoma this overdevelopment was probably more pronounced originally than was apparent at the time of death.

A study of the hypothalamus in approximately 200 cases suggests that a considerable amount of chromatolysis in the hypothalamic nuclei indicates the presence of a disease in which these nuclei are involved. In cases free from such disorders we find an average of not more than 6 to 8 per cent of chromatolytic cells in the nuclei tubero-mamillaris, substantia grisea, supraopticus, and paraventricularis. In various combinations of control groups 15 per cent of chromatolytic cells are commonly found in the nucleus tuberis lateralis.

In the patients suffering from carcinoma the average proportion of chromatolytic cells was 62.6 per cent for the nucleus tubero-mamillaris; 57 per cent for the nucleus tuberis lateralis; 36.7 per cent for the substantia grisea; 56.5 per cent for the nucleus supraopticus; and 55.8 per cent for the nucleus paraventricularis. This indicates that in carcinoma there is extensive involvement of all the nuclei of the hypothalamus.

In the material studied there was also evidence of widespread cell destruction in the hypothalamus. The tendency for some of the cases to have more than the normal number of cells in 4 of the nuclei made it difficult to estimate accurately the amount of cell destruction that had occurred. This factor, however, makes it probable that the amount of cell loss was greater than is shown by the cell count.

There was no apparent relationship between the origin or site of the tumor and the degree or pattern of hypothalamic involvement. The case histories in most instances did not permit a reliable estimate of the duration or the speed of growth and metastasis of the neoplasm. It is of interest to note, however, that in 5 of the cases, 4, 7, 13, 16, and 18, showing the most extensive metastasis the cell count for 4 of the hypothalamic nuclei was considerably higher than the average for the remainder of the group. The average count for the group with extensive metastasis was 122 per cent for the tubero-mamillaris; 85 per cent for the tuberis lateralis; 105.8 per cent for the supraopticus; and 85.2 per cent for the paraventricularis. For the remaining cases the average cell counts for the corresponding nuclei were 109.3, 75.8, 78.6, and 63 per cent.

It seems possible that the high count associated with pronounced chromatolysis may represent a state of hyperexcitability, and hence perhaps of hyperfunction, in these nuclei. We cannot exclude the possibility, however, that the greater amount of cell loss associated with the less malignant carcinomas may be due to the longer period of time during which the disease was active.

A striking feature of this study is that in carcinoma all 5 of the cell groups were found to be involved. As these are commonly thought to compose a central control and integrating mechanism for many, if not all, vegetative functions the involvement of all in carcinoma might well influence virtually every vegetative function, including the autonomic, endocrine, and metabolic.

The extensive studies carried out by many investigators on a wide variety of vegetative functions in both experimental cancer and in cancer patients have been reviewed by Stern and Willheim (12). On the whole they suggest abnormalities in oxygen metabolism; respiratory quotient; potassium:calcium ratio; and carbohydrate, protein, fat, and water metabolism. Virtually all the endocrine glands have been suspected by various investigators of playing a role in neoplastic growth and several, but not all, regard these functional deviations as systemic in character rather than confined to the tumor proper.

A survey of these functional studies seems to justify these general conclusions. (a) Widespread deviations in vegetative function occur in experimental animals and in patients afflicted with cancer. (b) These abnormalities are usually not of sufficient magnitude to make it certain that the deviation of any one function is of primary importance. (c) The deviations are subject to wide variation; they do not conform to a uniform pattern.

These conclusions are supported by the fact that in carcinoma there is an extensive but variable involvement of the central mechanism in the brain for the control and integration of vegetative functions. The present study does not enable us to say whether these cell changes in the hypothalamus are primary or secondary in nature.

The present study suggests the need for further research along certain lines. Most functional studies have been concerned with a single function in a large series of animals or patients, a type of study that is of fundamental importance and that has produced much valuable knowledge. However, it should be supplemented by an attempt to evaluate as far as possible the total complex of vegetative functions in the same indi-

vidual or group, since such an approach should help to evaluate the reaction of the total organism to cancer.

If a postmortem study of the hypothalamus could be repeated on a group of patients in which a more complete series of functional tests had been made it might lead to a better understanding of the relationship of the hypothalamus to the functional disorders occurring in cancer.

It would be of interest to make a comparative study of the hypothalamus in high tumor and low tumor strains of animals. This should indicate whether there is any congenital anomaly or histological alteration in the high tumor strain that might be considered as predisposing toward malignant growth. If the hypothalamus is involved in tumor-bearing animals, a study of the cell changes through the successive stages of tumor development might indicate whether these changes are primary or secondary.

SUMMARY

A histologic study was made of 5 nuclei of the hypothalamus in 19 patients with proved carcinoma.

Extensive chromatolysis and cell destruction indicated that all these cell groups are involved in carcinoma. The pattern of these changes showed a wide range of variation.

A congenital overdevelopment of some of the nuclei was indicated, but the cell destruction that occurs in carcinoma made it impossible to evaluate this factor properly.

The 5 nuclei studied are regarded as constituting a central mechanism for the control and integration of vegetative functions. This control is mediated largely through the autonomic and endocrine systems and influences most, if not all, metabolic functions.

The cell changes in the hypothalamus suggest a widespread but variable instability or irregularity of vegetative functions in the patient with carcinoma. This is in keeping with the findings of numerous investigators who have made functional studies in animals or human patients with cancer.

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Abstracts

Reports of Research

3:4-Benzpyrene from Coal Tar. BERENBLUM, I. [Oxford Univ. Research Centre of Brit. Emp. Cancer Campaign, Oxford, England] *Nature, London,* **156**:601. 1945.

The author has developed a process for obtaining 3,4-benzpyrene which "seems sufficiently promising for application of the method on a large scale as a practical means for supplying this important hydrocarbon." A crude distillate of tar (200 to 240° C./0.1 mm. Hg.) is extracted with concentrated sulfuric acid and the extract diluted with water and extracted with benzene; the benzene extract is washed and dried, adsorbed on and eluted from alumina, and the eluate is dried and recrystallized. Ten grams of tar distillate gave 75 mgm. of almost pure benzpyrene. A quantitative test on a tar distillate to which was added pure benzpyrene gave almost 100% recovery.—I. H.

The Effect of Various Solvents on the Rate of Elimination and Carcinogenic Activity of 3:4-Benzpyrene. DICKENS, F., and WEIL-MALHERBE, H. *Biochem. J.,* **39**:xxxix. 1945.

It has previously been shown that fat from the same species of animal when used as a solvent for benzpyrene inhibits carcinogenesis; this effect can, however, be reproduced by phosphatides (lecithin, cephalin) from other species. It has now been found that cholesterol strongly enhances carcinogenic activity. Hydrogenated fats and cod liver oil (rich in polyethenoid fatty acids) were not anticarcinogenic.

"The rate of elimination of benzpyrene from mice was measured by its chromatographic separation and fluorimetric estimation. By comparison with pure tricaprylin, elimination was significantly accelerated by the presence of dissolved cholesterol and delayed by the phosphatides. The high tumour incidence with the former, and the low incidence with the latter are opposed to the view that slow elimination favours carcinogenesis. It is presumed that this effect is primarily connected with the rate of oxidative metabolism of the carcinogen, which is depressed by phosphatides, probably through their antioxidant properties, and accelerated by cod liver oil and cholesterol in varying degrees. Up to a certain limit, the rapid metabolism of the hydrocarbon favours carcinogenesis, while depression of carcinogen metabolism is anticarcinogenic. This would accord with the hypothesis that the hydrocarbon is not itself the true carcinogen, but that the active formation of an oxidized metabolite determines the activity. In this case the metabolite of the hydrocarbon must be considered as the true carcinogen." [The complete data are not included in the summary from which this abstract was made.]—I. H.

Distant Tumours Produced by 2-Amino- and 2-Acetyl-Amino-Fluorene. BIELSCHOWSKY, F. [Univ. of Sheffield, Sheffield, England] *Brit. J. Exper. Path.,* **25**:1-4. 1944.

No spontaneous tumor or leukemia has been observed during the last 4 years in the strain of Wistar rats used; mammary cancer could be produced in them by implanting stilbestrol pellets under the skin. 2-Acetylaminofluorene (4 mgm. daily) was given in the food to 104 rats, 93 of which developed malignant tumors; of these 34 hepatomas and 3 mammary cancers occurred in uncastrated males, and 11 hepatomas and 23 mammary cancers occurred in uncastrated females. Nearly all the animals showed cystic cholangiomas, which were not regarded as malignant. Only 1 out of 11 spayed rats developed mammary cancer. 2-Acetylaminofluorene has no estrogenic activity. "An unusual type of tumour which was observed in 16 instances is a carcinoma arising from the ductus acousticus externus. It originates in the sebaceous glands of the duct, or starts as malignant papilloma from the squamous epithelium." These tumors occurred in both sexes with about the same frequency. Five adenocarcinomas of the gut occurred. The hepatomas frequently formed metastases in the lung, as did the carcinomas of the ductus acousticus in 3 cases. Three liver tumors and 3 breast cancers were transplanted. One spayed rat developed leukemia; subcutaneous injection of the blood in 3 young rats produced rapidly growing tumors and later, a leukemic blood picture. Intravenous injection of such blood or of tumor cells produced leukemia and local tumors.

Most of the tumors mentioned above appeared between the 180th and 280th day. Five male rats were painted with 2-aminofluorene in acetone; after 280 days all the animals showed malignant hepatoma, and 1 of them had also a carcinoma of the ductus acousticus. There was no alteration in the painted skin.—E. L. K.

Glioma in a Rat Fed with 2-Acetyl-amino-fluorene. VAZQUEZ LOPEZ, E. [Imp. Cancer Research Fund, London, England] *Nature, London,* **156**:296-297. 1945.

A glioma is reported in 1 of 12 rats that were fed from the age of 2 months, for a 24 week period, on a diet containing 0.5 gm. of 2-acetylaminofluorene per kgm. of food. The animal died 2 weeks after incoordination of movement and a slight paresis were first observed. Postmortem examination revealed asymmetry of the cerebral hemispheres, with the left enlarged frontally. Sections showed an infiltrating neoplastic growth extending throughout the olfactory bulbs, the white matter of the frontal and temporal lobes, and the anterior and main part of the lateral ventricle. It also invaded the lepto-

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meninges. The tumor was diagnosed histologically as a glioma of the type "glioblástoma isomorphe" of Rio-Hortega.—R. J. L.

Experimental Nodular Goitre. Bielschowsky, F. [Univ. of Sheffield, Sheffield, England] *Brit. J. Exper. Path.*, **26**:270-275. 1945.

A continuation of an earlier investigation (*ibid.*, **25**:90. 1944; abstract in *Cancer Research*, **5**:56. 1945) upon the simultaneous administration by mouth of 2-acetylaminofluorene and allyl-thiourea, in which benign and malignant tumors of the thyroid were produced. The administration of 2-acetylaminofluorene followed by allyl-thiourea produced multiple adenomas of the thyroid, but no malignant tumors, while allyl-thiourea alone produced single adenomas only.—E.L.K.

Studies on Experimental Goiter. VI. Thyroid Adenomata in Rats on Brassica Seed Diet. Griesbach, W. E., Kennedy, T. H., and Purves, H. D. [Thyroid Research Dept. of New Zealand Med. Research Council, Univ. of Otago, Dunedin, New Zealand] *Brit. J. Exper. Path.*, **26**:18-24. 1945.

Prolonged continuous thyroid hyperplasia produced by the goitrogenic agent in *Brassica* seeds leads to the formation of thyroid adenomas.—E.L.K.

Effect of Nucleates on Dehydrogenase Systems. Chalkley, H. W., and Greenstein, J. P. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **6**:119-141. 1945.

Studies were made of the effects of yeast and thymus nucleates on the decolorization rate of methylene blue in tissue extracts. The effects of the added nucleates varied with the conditions of the experiments when normal liver extracts were employed. At low dye concentrations both types of nucleate depressed the rate of decolorization, while at relatively high dye concentrations an acceleration of decolorization resulted; at intermediate concentrations of dye yeast nucleate accelerated, whereas thymus nucleate depressed, the decolorization rate. When extracts of hepatoma were studied, however, an accelerating effect of both nucleates over a wide range of dye concentration was noted. With the addition of oxidizable substrate such as xanthine, the point at which the effect of added nucleate changed from depression to acceleration was shifted in the direction of higher dye concentration. Increasing concentrations of nucleates accentuated the depressant effects; decreasing concentrations, in the absence of added substrate, yielded maximum acceleration. Xanthine dehydrogenase activity was calculated in several mixtures and, although found to be dependent upon dye concentration, appeared to be augmented by the addition of nucleates. Coenzyme I was "found to be responsible in a considerable measure" for the decolorization of methylene blue, but it was found that this factor was active only in the presence of the bicarbonate ion. The implications of the various findings were discussed.—R.A.H.

Cell Proliferation, Carbohydrate Breakdown, and Hydration of Enzyme Protein. Lasnitzki, A. [Univ. of Birmingham, Birmingham, England] *Nature, London*, **156**: 398. 1945.

Rapidly proliferating tissue (embryonic and neoplastic) contains more water than adult and normal tissue; ions that favor hydration of proteins also accelerate glycolytic activity of normal tissues and tumors (inhibiting ions have the opposite effect), and finally, increased glycolytic activity is closely connected with rapid proliferation. In regard to this self-contained scheme the author states: "In all probability, the increase in tissue water is due in great part to a rise in water content of the cellular constituents, and this rise is likely to be associated with an increased hydration of cell proteins. The most important kinds of proteins, in this connexion, appear to be those which constitute the protein components of enzymes, and, in particular, of such enzymes as are involved to a varying extent in the breakdown of carbohydrates.

"These considerations suggest, therefore, that the intensity of carbohydrate breakdown in a growing tissue, and consequently the rate of cell proliferation, depends largely on the degree of hydration of corresponding enzyme proteins, so that, within limits, an increase in hydration stimulates and a decrease inhibits that enzymatic activity. The suggestion attempts to elucidate the biological significance of the increased water content of rapidly growing tissues by relating it, through the concept of protein hydration, to the metabolic process which serves as the source of energy for cell proliferation. . . ."—I. H.

The Growth of Mammalian Tumors in Fertile Eggs. Is a Filterable Cancer Virus Produced? Twombly, G. H., and Meisel, D. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Cancer Research*, **6**:82-91. 1946.

Rat sarcoma 39, Bagg mouse carcinoma 755, and the RC mouse carcinoma of Taylor were grown successfully in fertile incubated hen's eggs. The mortality of the eggs was very high, 73% by the 17th day of incubation, making this method a poor one for the routine growth of tumor tissue. The yolk from eggs bearing the rat sarcoma 39 did not produce sarcomas when injected into susceptible rats save in one instance in which the centrifuged sediment of such yolk was effective. Injection of such yolk did not confer immunity on young rats. Yolk from eggs bearing either of the two mouse mammary carcinomas frequently reproduced the tumor when injected into susceptible mice. It was equally effective in male and female mice. Repeated attempts to filter the tumor agent through Berkefeld N or V candles were unsuccessful. Freezing and thawing of yolk, or lyophilization destroyed tumor-producing activity, as did heating at 48 to 50° C. for 30 minutes. If yolk from tumor-bearing eggs was diluted 1:1 with saline and centrifuged at low speed (2,600 r. p. m.) for 5 minutes, tumor activity could be demonstrated almost universally in the fatty cream found above the centrifuged yolk and in the bloody sediment at the bottom, but not in the watery yolk between. The tumor-producing layers contained cells easily demonstrable under the microscope. Tumor-producing activity was not closely correlated with red blood cell content nor could it be released by hemolysis from sediments containing red cells. Most of the tumor-producing activity could be removed by simple filtration through filter paper. Tumor-producing activity of the fatty layer was destroyed by extraction with ether. In the authors' opinion, the tumor-producing capacity of egg yolk from yolk sacs in which mammalian tumors have

been grown is due to the presence in it of viable tumor cells. No convincing evidence of the presence of a virus or filterable agent has been encountered in their experiments.—Authors' summary.

The Respective Roles of Longevity and Genetic Specificity in the Occurrence of Spontaneous Tumors in the Hybrids between Two Inbred Lines of Rats. Dunning, W. F., and Curtis, M. R. [Wayne Univ. Coll. of Med., and Detroit Inst. for Cancer Research, Detroit, Mich.] *Cancer Research,* **6**:61-81. 1946.

Analysis of the occurrence of spontaneous tumors in the first 10 brother-by-sister generations of two lines of rats showed one to be long-lived with a relatively high incidence of tumors and the other relatively short-lived with few spontaneous neoplasms. The first 10 brother-by-sister generations of Copenhagen line 2331 had an average life span of 19.6 ± 0.13 months, and 450 neoplasms were observed in 20% of the rats that survived for at least 1 year. Of these growths, 80% involved the thymus gland. Rats of the first 10 brother-by-sister generations of the Fischer line 344 survived an average of 12.8 ± 0.11 months, and only 42, or 1.7%, of those rats that survived for 8 months developed tumors, of which 20 were mesenteric lymph node sarcomas, and none involved the thymus gland.

The 11th to 20th brother-by-sister generations of these lines, which were the contemporaries of the reciprocal F_1 and backcrossed hybrids, showed the same respective predominance of tumors of the thymus gland and mesenteric lymph nodes, but showed for each a significant reduction in the average life span. These Copenhagen line 2331 rats lived an average of 16.2 ± 0.16 months, and the Fischer line 344 rats an average of 10.4 ± 0.09 months.

Reciprocal F_1 hybrids between these two inbred lines had a longer average life span than their contemporaneous relatives of either parent line. Progeny of Fischer line 344 males and Copenhagen line 2331 females lived an average of 17.7 ± 0.43 months, and the reciprocal hybrids 20.9 ± 0.35 months. No thymic neoplasms were observed in the F_1 hybrids, and the incidence of mesenteric lymph node sarcomas was lower than that observed for Fischer line 344 rats. The percentage of tumors involving miscellaneous organs and tissues was greater than that observed for the low tumor parent line. There was no evidence of the maternal transmission of either susceptibility or resistance to the common laboratory diseases.

Progeny of the F_1 hybrids backcrossed to the long-lived higher tumor line lived longer and had a significantly higher tumor incidence than the progeny of these hybrids that were backcrossed to the low tumor short-lived line. Mesenteric lymph node sarcomas were observed in all groups of backcrossed hybrids in a proportion not significantly different from that observed in the Fischer line 344 parent line. Thymic neoplasms were observed in 3 of the 4 categories of backcrossed hybrids but in a significantly lower percentage of rats than was observed for the Copenhagen line 2331 or thymic tumor susceptible parent line.

Spontaneous neoplasms involving miscellaneous organs and tissues occurred in both parent lines and in all the hybrid groups. Longevity and hybrid vigor seemed to be potent influences in the incidence of these neoplasms.—Authors' abstract.

Behavior of Ultimobranchial Tissue in the Postnatal Thyroid Gland: Epithelial Cysts, Their Relation to Thyroid Parenchyma and to "New-Growths" in the Thyroid Gland of Young Sheep. Van Dyke, J. H. [Cornell Univ., Ithaca, N. Y., and Washington Univ. Sch. of Med., St. Louis, Mo.] *Am. J. Anat.,* **76**: 201-252. 1945.

Ultimobranchial tissue occurs as cysts in the thyroids of young sheep. The stratified squamous epithelium lining these cysts may develop in two directions. In active thyroids the undifferentiated clear cells of the basal layer may transform into typical thyroid-like tissue. On the other hand, in hyperplastic and atrophic thyroids the more dense suprabasal cells of the cyst epithelium transform into aberrant thyroid tissue, which in some cases forms conspicuous adenoma-like masses.—R. B.

Paneth Cells in Carcinomas of the Small Intestine in a Mouse and in a Rat. Dunn, T. B., and Kessel, A. M. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **6**:113-117. 1945.

The histological characteristics of 2 tumors of the small intestine, one occurring in a mouse and the other in a rat, are given. Both tumors contained numerous Paneth cells, and the staining reactions of these elements were studied and compared with those of normal Paneth cells. Ten tumors of the small intestine were found recorded in the files of the National Cancer Institute, and Paneth cells were noted in 7.—R. A. H.

The Injurious Effect of Light upon Dividing Cells in Tissue Cultures Containing Fluorescent Substances. Lewis, M. R. [Carnegie Institution of Washington, Baltimore, Md., and Wistar Inst., Philadelphia, Pa.] *Anat. Rec.,* **91**:199-208. 1945.

Embryonic cells of the chick were grown in culture media containing the following fluorescent chemicals: chlorophyll, dibenzanthracene, and methylcholanthrene, each in a concentration of 1 to 50,000; and neutral red and eosin, each in a concentration of 1 to 100,000. The cells divided and grew normally in the dark. But when exposed to a bright light the dividing cells became abnormal, while resting cells remained undamaged and were able to resume normal mitotic activity if returned to the dark. The injury to the dividing cells consisted in a shortening of the spindle and an agglutination of the chromosomes on the equatorial plate. In addition mitochondria and other granules moved into the space previously occupied by the spindle; and on the surface of the cell many blebs appeared.

Eosin and neutral red acted on the cells more rapidly than did chlorophyll or the carcinogens.—R. B.

Colchicine in the Experimental Chemotherapy of Cancer. Ludford, R. J. [Lab. Imp. Cancer Research Fund, Mill Hill, England] *J. Nat. Cancer Inst.,* **6**:89-101. 1945.

The literature is reviewed regarding the mode of action of colchicine, and the chemotherapeutic results obtained from its use in the treatment of cancer in experimental animals and in man. In extremely low concentration this drug acts as a mitotic poison inhibiting the formation of the spindle; in somewhat greater concentration it may also cause a distortion of the chromosomes. When tumor-bearing animals are injected with the highest tolerated

doses of colchicine, hemorrhage and necrosis similar to those seen following the injection of certain bacterial filtrates are noted within the tumors. Some very striking results have been reported from the use of colchicine in the treatment of malignant growths in experimental animals; in some instances complete regression of the lesions resulted. In general, large doses were employed, much larger than required for mitotic poisoning and in the range causing capillary damage. Tumors that responded to colchicine therapy were of types that are also radiosensitive, and, by means of x-ray and colchicine combined, therapeutic results superior to those obtained by either method alone have been obtained. The results of therapy in a limited number of human cases have not been as successful as might have been hoped for on the basis of animal experimentation. Sixty references.—R. A. H.

Significance of Negative Results in Small Samples. Levin, M. L., and Goldstein, H. [New York State Dept. of Health, Albany, N. Y.] *Science*, **102**:407. 1945.

The custom of using only 10 to 25 animals in testing chemicals for therapeutic effects on cancer is criticized. While positive results with small numbers are significant, negative results may not be. Thus, on the basis of probability statistics it is shown that tests done with groups of 10 animals might fail to detect the therapeutic effects of chemicals having a true effectiveness of less than 25%. Similarly, tests done with groups of 28 animals might fail to detect as therapeutic, chemicals with less than 10% true effectiveness.—R. B.

Electroencephalogram of Dogs with Experimental Space-Occupying Intracranial Lesions. Ulett, G. [Univ. of Oregon Med. Sch., Portland, Oreg.] *Arch. Neurol. & Psychiat.*, **54**:141-149. 1945.

Experimental (foreign body), subcortical, space-occupying lesions in dogs produced high voltage, slow (delta) waves in the electroencephalogram, changes that resembled the electroencephalographic alterations seen in some cases of intracerebral space-occupying lesions in man. Normal rapid activity disappeared, and flattening of waves occurred with subdural and extradural space-occupying lesions. The amount of abnormality can be varied by minor shifts in electrode placement in cases of focal damage to the brain.—M. E. H.

Relationships of Lymphocytes and Cancer. Kellsall, M. A. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] *Science*, **102**:456-457. 1945.

This is a short review of the literature on lymphocytes and cancer, in which it is pointed out that cancer tissue and sites of action of carcinogenic stimuli frequently contain increased numbers of lymphocytes, while conditions (*e.g.*, inanition, radiation) that reduce the growth and incidence of tumors also reduce the number of circulating lymphocytes.—R. B.

Cytoplasmic Diseases and Cancer. Woods, M. W., and duBuy, H. G. [Indust. Hyg. Research Lab., National Inst. of Health, Bethesda, Md.] *Science*, **102**:591-593. 1945.

A statement of a theory relating viruses and pathogenic plant mitochondria (plastids) to hypothetical cancer-inducing mitochondria.—R. B.

William Cramer (1878-1945). Woglom, W. H. *Cancer Research*, **6**:30-35. 1946.

A brief biographical sketch, with portrait photograph and a list of publications from 1902 to 1945.—M. H. P.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

Therapy—General

Calcium et métallothérapie dans le cancer. [Calcium and Metallotherapy in Cancer.] Vassiliadis, H. *Bull. Assoc. franç. p. l'étude du cancer*, **29**:192-196. 1940-1941.

Since 1931, metallotherapy has been employed in the treatment of all cancer patients in the clinic of Professor J. Maisin and found (data published elsewhere) to be effective alone and as an adjuvant to other forms of therapy.

Observations on 20 patients maintained on a low calcium diet showed that when barium saccharate was given orally (10 drops of 1:10,000 dilution every other day) the urinary excretion of calcium was increased. Therefore, cancer patients being treated with barium saccharate are now given supplementary calcium to compensate for this calcium loss.—G. H. H.

Etiology

Ischemia as Cause of Cancer. Kullberg, R. W. [Astoria, Oreg.] *Northwest Med.*, **44**:107-110. 1945.

This is an interpretation of data quoted from the literature in support of the author's thesis that ischemia is an important intrinsic cause of cancer.—E. E. S.

Radiation

Further Problems in X-Ray Protection. I. Radiation Hazards in Photofluorography. Birnkrant, M. I., and Henshaw, P. S. [U. S. Pub. Health Service, Washington, D. C.] *Radiology*, **44**:565-568. 1945.

Scattered radiation measured near the surface of the skin was found to be 18 times greater with 35 mm. photofluorographic technic than with 14 by 17 inch film technic. The person examined was the chief source of scatter. Sufficient radiation to exceed safety limits (0.1 r daily) can be received, if the operator is careless. Behind lead screens the margin of safety is not exceeded. An extension cone to the x-ray tube reduces stray radiation.—R. E. S.

Further Problems in X-Ray Protection. II. Irradiation Injury and the Tolerance Dose. Henshaw, P. S. [Nat. Cancer Inst., Bethesda, Md.] *Radiology*, **44**:569-580. 1945.

The types of irradiation injury encountered after ex-

posure to radiation are dermatitis and carcinoma, sterility, anemia and leukemia, mutations, genetic injury, and shortening of the life span. For skin changes, sterility, and aplastic anemia, it seems clear that there are threshold dosage levels below which no changes are produced. However for mutations, the evidence seems to indicate that there are no thresholds and that we cannot be sure of a safe tolerance dose. For practical purposes some safety level must be accepted, and the present standard of 0.1 r per day seems reasonable. In the absence of more complete information, it is felt that our efforts should be directed toward maintaining this standard. Sixty-two references.—R. E. S.

Further Problems in X-Ray Protection. III. Protective Measures in Photofluorography. Birnkrant, M. I., and Henshaw, P. S. [U. S. Pub. Health Service, Washington, D. C.] *Radiology,* **44**:581-584. 1945.

The protective measures used by the Tuberculosis Control Division of the United States Public Health Service in photofluorographic units consist in careful planning of location of equipment; provision of adequate protective equipment, including a 1.5 mm. lead screen for the operator; detection, by the use of dental film, of stray radiation reaching workers; and monthly blood counts.—R. E. S.

Results of Irradiation of Ovarian Tumors. Kerr, H. D., and Einstein, R. A. J. [Coll. of Med., State Univ. of Iowa, Iowa City, Iowa] *Am. J. Roentgenol.,* **53**:376-384. 1945.

Five year data are presented on 100 consecutive cases in which a diagnosis of ovarian tumor was made. All were treated by combinations of surgery and irradiation; in general irradiation is used after surgical removal of as much neoplastic tissue as possible. Maximum tumor dose of radiation is sought, regardless of clinical or pathological classification. Multiple fields around pelvis and abdomen are employed, and most patients received more than 2,000 r to the tumor. Five year survival rates were 76% after surgical removal of the primary tumor and all visible metastases, 38% after partial or total removal of the primary growth but with visible metastases remaining or with ascites or spill of malignant cells into the peritoneal cavity, 12% in instances of recurrence following surgery or irradiation, 3% in cases of inoperable primary growth or distant metastases, and 0% in an unclassified group. The total 5 year survival rate among the 95 patients with malignant ovarian tumors was 40%.—E. H. Q.

Changes in the Uterus after Eradication of Endometrial Adenocarcinoma by Radiotherapy, with Particular Reference to an Infarct-Like Radionecrotic Plaque in the Lining. Sheehan, J. F., Schmitz, H. E., and Towne, J. [Loyola Univ. Sch. of Med., and Mercy Hosp., Chicago, Ill.] *Arch. Path.,* **39**:237-256. 1945.

A thorough gross and microscopic study was made of 4 uteri excised after eradication of carcinoma of the endometrium by large doses of radium (about 6,000 mgm.-hr.) and of roentgen radiation (about 4,000 r in the midpelvis). Two other irradiated uteri were taken into consideration, although only routine sections of these were available. A carcinoma of the endometrium in one of these was destroyed by radiation.

The original site of the carcinoma in 5 of the 6 uteri could not be determined. A localized plaque-like area of radionecrosis, essentially an area of coagulation necrosis, was found in the lining at or near the level of the internal os in 5 of the uteri. Changes were produced in the plaque by hemorrhage and infection. There is a question whether or not plaques of the type observed are true infarcts. In the myometrial tissues adjacent to the uterine plaque two zones showing the effects of radiation were found: a superficial zone of hyalinization and edema with necrobiotic changes and a deeper zone of edema with atrophic changes. In the cervix a single zone of hyalinization was the usual finding. Vascular changes were encountered in these zones but were not confined to them. Other observations included some degree of chronic cervicitis and endometrial atrophy; chronic metritis, mild and more or less focal; and other nonspecific lesions, including squamous metaplasia of the endometrial epithelium in 1 case.—Authors' summary. (J. G. K.)

Discussion on Rationale of Radiotherapeutic Technique in Carcinoma of the Larynx. Dobbie, J. L., et al. *Proc. Roy. Soc. Med.,* **38**:348-352. 1945.

(The published data are abstracts of the papers read, and the originals should be consulted.)

Dobbie, J. L. An analysis of a large group of treated cases showed that while 29% of patients without lymph node involvement survived 5 years, only 4% of those having such involvement did so. In practice a continuous sequence from large extrinsic to small intrinsic lesions is found. The larger pharyngolaryngeal tumors can be treated only by external irradiation; beam-directed therapy shows its best results when fields of about 6 cm. diameter can be used. For fields smaller than 6×4 cm. it is thought that the tumor can be better treated by technics resulting in less volume dosage. These technics involve operative exposure. The Finzi-Harmer technic is the most localized of all and should be used only for a small tumor isolated on a mobile cord and toward the middle of it. It is so far the most successful technic.

Adams, S. B. The importance of the time-dose factor in treatment of neoplasms of the larynx is stressed. As an alternative to fenestration, a "skin-reflection method" has given encouraging initial results. A reasonably large dose of x-radiation is given through one or two portals. Glandular metastases are locally excised when the skin flap is made.

Lederman, M. The technic for treatment of intrinsic cancer of the larynx by teleradium therapy is described. The tumor dose varies considerably from case to case, ranging from 5,000 to 10,000 r, with 7,000 r as an average. The integral dose is low. The conduct of the treatment depends on the response of the patient, the tumor, and the normal tissues subjected to irradiation. The main object is to obtain a heterogeneous field of radiation with maximal dose at the tumor site.

Wade, P. A series of cases of carcinoma of the larynx was treated by a technic involving both γ-rays and x-rays. In many cases γ-rays are most suitably administered by means of the radium beam, the dose being raised by the addition of an x-ray field in a central posterior position. For success the vertical limits of the growth must be very circumscribed.

JOLLES, B. Neoplastic tissues in the larynx respond differently to radiation in the presence or absence of edema in or around the growth. Soft tissue radiography complementary to laryngoscopy is of great value if carefully undertaken.

FINZI, N. S. A presentation of gramophone records of the voice in cases of laryngofissure and radium implantation showed the almost normal voice obtained after the radium method as compared with even the good laryngofissure voices.

SIMCHOWITZ, H. C. A new method of intralaryngeal contact therapy, described by Chaoul, employs a modified contact therapy tube fixed to a specially designed direct laryngoscope.

ELLIS, F. The principles to be observed in treating carcinoma of the larynx by radiation would appear to be: (a) economy of radiation; (b) accuracy of application; (c) choice of the best time-dosage relationship; (d) avoidance of the ill effects of sepsis.

KOLLER, P. C. In spite of considerable individual variations in time of appearance and degree of erythema, the conclusion that a higher dose is required to produce erythema by γ-radiation than by x-rays has been verified by many investigators. It is necessary to distinguish between radiation effects induced in (1) single cells, and (2) tissues. The low dosage rates employed in γ-ray therapy may make this method better suited than x-rays to the treatment of some tumors, among which can be included tumors of the larynx.—W. V. M.

NERVOUS SYSTEM

Intracranial Vascular Tumors and Malformations. NORAN, H. H. [Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Arch. Path.*, **39**:393-416. 1945.

General review.—J. G. K.

Diffuse Meningeal Fibroblastoma of the Brain and Spinal Cord—A Report of Three Cases. HAYTHORN, S. R., SHAPERA, W., and STEWART, H. C. [Allegheny Gen. Hosp., Pittsburgh, Pa.] *Arch. Path.*, **39**:287-293. 1945.

Mallory's phosphotungstic acid-hematoxylin stain disclosed fibroglia and collagen fibers, and these served to identify the tumor cells as fibroblasts in 2 of the 3 cases, thus furnishing additional evidence of the fibroblastic nature of meningiomas.—J. G. K.

Spinal Extradural Arachnoid Cyst Associated with Extradural Malignancy. COHEN, I. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **12**:116-118. 1945.

Multiple spinal cord tumors are rare. A case is reported in which the patient had an extradural neuroblastoma (sympathicoblastoma) at the tenth thoracic vertebra and an extradural arachnoid cyst at the eighth. The latter is an unusual intraspinal lesion, especially infrequent in adults.—A. Cnl.

BREAST

The Therapy of Breast Carcinoma. HERRMANN, J. B. [Memorial Hosp., New York, N. Y.] *Connecticut M. J.*, **9**:178-184. 1945.

Radical mastectomy in properly selected cases produces the highest percentage of 5 year survivals of any therapeutic procedure for breast cancer. There are, however, definite contraindications to surgery. Preoperative irradiation does not increase the number of 5 year survivals; postoperative irradiation in patients with anaplastic carcinoma or with axillary node involvement is distinctly advantageous, but x-ray therapy should not be relied upon to supplement incomplete surgery. Castration either by x-ray or surgery is of value in about 15% of cases. In inoperable or metastatic carcinoma of the male breast, surgical castration is the method of choice. Androgens in the treatment of carcinoma of the female breast are of questionable value. Pregnancy associated with mammary cancer makes the prognosis unfavorable and should be terminated. Heptaldehyde and H 11 have been disappointing as therapeutic agents in human mammary cancer.—M. E. H.

MALE GENITAL TRACT

The Cure of Cancer of the Prostate by Radical Perineal Prostatectomy (Prostato-Seminal Vesiculectomy): History, Literature, and Statistics of Young's Operation. YOUNG, H. H. [Johns Hopkins Hosp., Baltimore, Md.] *J. Urol.*, **53**:188-252. 1945.

This is a comprehensive review of the author's experiences in developing, perfecting, and applying perineal prostatectomy for the cure of carcinoma. Reasons for the various steps of the operation are discussed. At the Brady Institute, 184 radical operations have been done, with 12 deaths in the hospital. Each death is discussed. Pathological studies indicate that invasion outside the prostate is a late manifestation; the rectal mucosa is only rarely invaded; the tissues under the trigone and the seminal vesicles are usually invaded first. Metastases are most common in the bones of the pelvis, spine, and femur. The author believes that metastases are apparently more common by way of the blood stream than by the lymphatics. Approximately 50% of the patients with carcinoma also had benign hypertrophy. Most of these cancers begin in the posterior lamella, and hence even removal of the periurethral benign hypertrophied mass may not disclose neoplasia when the cancer is still early and suitable for cure by radical operation. The need for careful attention to small hard areas in the prostate is demonstrated by diagrams of the rectal findings of the 38 patients regarded as cured by this operation for 5 or more years. These 38 cases are described in some detail. Statistics show that prostatic cancer occurs in at least 14% of all men more than 44 years of age, and that it is three times as common as cancer of any other internal organ in males. The cure rate by Young's radical prostatectomy, where feasible, is apparently nearly 50%.—V. F. M.

Indications for Bilateral Orchiectomy in the Treatment of Carcinoma of the Prostate. MEADS, A. M. [Oakland, Calif.] *J. Urol.*, **53**:415-418. 1945.

The author reviews the answers of 78 members of the American Urological Association to a questionnaire on this subject. Seventy favored orchidectomy, 1 was doubtful, and 7 did not use the operation. There was wide

divergence of opinion regarding the time during the course of the disease at which orchidectomy should be done. It was most constantly advocated for late cases.—V. F. M.

Early or Late Orchiectomy for Carcinoma of the Prostate. ALYEA, E. P. [Duke Univ. Sch. of Med., and Duke Hosp., Durham, N. C.] *J. Urol.*, **53**:143-153. 1945.

A comparison of the survival rates for 110 patients with prostatic cancer treated by orchidectomy, with the survival in 1,000 cases of prostatic cancer reported by Bumpus in 1926, indicates a definite prolongation of life by castration. Twenty-six patients without signs or symptoms of metastases at the time of orchidectomy continue without such evidence for more than 1 year postoperatively. Orchidectomy plus stilbestrol is suggested as the therapy of choice for cases not suitable for radical prostatectomy.—V. F. M.

Carcinoma of the Prostate Gland. PALOMO, A. [New York Hosp., New York, N. Y.] *J. Urol.*, **53**:166-187. 1945.

Of 165 patients with prostatic carcinoma treated from 1921 to 1938, principally by surgery plus radiation, 18 are known to have survived 3 years or longer. Of 44 treated by prostatectomy or resection between 1938 and 1944, 13 lived 3 years or more; and 20 of 47 treated by castration during the same period were alive on January 1, 1945. Surgical technics are described and illustrated.—V. F. M.

Carcinoma of the Prostate. HOWARD, J. C. [New York, N. Y.] *Urol. & Cutan. Rev.*, **49**:272-275. 1945.

Only prostatic cancer outside the capsule causes an elevation of blood acid phosphatase. One case, with radiation of the testes and stilbestrol therapy, is described in detail. When the disease is confined within the capsule, radical prostatectomy should give at least a 50% survival.—V. F. M.

An Analysis of 40 Cases of Carcinoma of the Prostate. STIRLING, W. C. [Washington, D. C.] *J. Urol.*, **53**:154-159. 1945.

By means of resection, castration, and estrogenic therapy, singly or in combination, 57% of the group reported survived an average of 16 months. Castration and estrogen treatment seemed to be palliative only. The relief of pain usually lasted until death. A high acid phosphatase level in the blood indicates osseous metastases, but low findings are of little diagnostic value.—V. F. M.

Treatment of Carcinoma of the Prostate Gland; a Comparative Study. CRANE, J. J., and ROSENBLOOM, D. [Los Angeles Co. Gen. Hosp., Los Angeles, Calif.] *J. Urol.*, **53**:411-414. 1945.

A review of 340 cases of treated prostatic carcinoma indicated that results were better with castration alone than with estrogens alone. Best results were obtained by thorough transurethral resection combined with castration plus continuous administration of stilbestrol.—V. F. M.

Side Effects Caused by Diethylstilbestrol and Correlated with Cancer of the Prostate Gland. WATTENBURG, C. A., and ROSE, D. K. [Washington Univ. Sch. of Med., and Barnes Hosp., St. Louis, Mo.] *J. Urol.*, **53**:135-142. 1945.

Thickening of the prostatic urethral mucosa, hypertrophy of the verumontanum, breast enlargement, and hypertrophy of the appendix testis are discussed and demonstrated as side effects of estrogenic treatment of prostatic carcinoma. The edema that occasionally occurs during treatment was found to be largely due to decreased renal excretion of sodium and chloride. These side effects offer no index of the degree of control of the cancer.—V. F. M.

Adenomatoid Tumors of the Genital Tract. GOLDEN, A., and ASH, J. E. *Am. J. Path.*, **21**:63-79. 1945.

Report of 15 cases seen during 2 years. The growths were characterized by a well-defined glandular pattern. Confined to the epididymis, testicular tunics, or serosal surface of the fallopian tube, they exhibited neither local tissue invasion nor metastases.—J. G. K.

Diagnosis and Treatment of Tumors of the Testis. HELLWIG, C. A. [St. Francis Hosp., and Sedgwick Co. Tumor Clin., Wichita, Kans.] *J. Kansas M. Soc.*, **46**:37-40. 1945.

Fifty malignant tumors (solid carcinoma, adenocarcinoma, and choriocarcinoma) were found on histological examination of 254 testicular specimens from patients with clinical diagnoses of tumor of the testis, during the past 20 years. Of 36 patients who were followed after treatment by orchidectomy, irradiation or both, 17 were dead and 19 alive at the time of writing. Of the patients treated before July 1939, 38.9% were alive. The results in cases of solid carcinoma and adenocarcinoma failed to support the view that the former is more radiosensitive. Aspiration biopsy for diagnosis is condemned as dangerous, and immediate total removal of possibly malignant testicular tumors is urged.—C. W.

Rhabdomyosarcoma of Testicle—A Case Report. BEARD, D. E., and HEWIT, L. W. *J. Urol.*, **53**:344-346. 1945.

A case report with illustrations.—V. F. M.

URINARY SYSTEM—MALE AND FEMALE

Wilms' Tumor in the Isthmus of a Horseshoe Kidney. ROSE, D. K., and WATTENBERG, C. A. [Washington Univ. Sch. of Med., and Barnes Hosp., St. Louis, Mo.] *Urol. & Cutan. Rev.*, **49**:365-367. 1945.

A Wilms' tumor, found in the isthmus of a horseshoe kidney, was removed by resection of the isthmus. This is the second reported instance of Wilms' tumor in a horseshoe kidney.—V. F. M.

The Relationship of Epithelial Buds to Carcinoma of Pelvis of the Kidney, Ureter and Bladder. BATHE, A. E. [Univ. of Pennsylvania, Philadelphia, Pa.] *J. Urol.*, **53**:451-458. 1945.

A microscopic study of ureters from 54 autopsies, and of surgical specimens from 12 patients with papillary carcinoma of the renal pelvis, ureter, and bladder has shown that epithelial cell nests are frequently present in these organs. The surgical specimens indicate that the early changes that first involve the subepithelial supporting tissues are inflammatory in type. Possibly it is the immature epithelial buds that are susceptible to the action of carcinogens. It is suggested that x-ray therapy may reduce their susceptibility: recurrences from low grade multiple small papillomas of the bladder occurred in 2 patients

treated by transurethral desiccation alone, but not in 2 treated by x-ray followed by this procedure.—V. F. M.

Mucinous Carcinoma of the Urachus Invading the Bladder. Hayes, J. J., and Segal, A. D. [Coney Island Hosp., Brooklyn, N. Y.] *J. Urol.*, **53**:659-669. 1945.

This is a detailed case report with a tabulation of 44 cases from the literature.—V. F. M.

Intrathoracic Tumors—Lungs—Pleura

Difficulties in the Differential Diagnosis of Bronchogenic Carcinoma. Bloch, R. G., Adams, W. E., Thornton, T. F., and Bryant, J. E. [Frank Billings Clin., Univ. of Chicago, and Provident Hosp. Clin., Chicago, Ill.] *J. Thoracic Surg.*, **14**:83-97. 1945.

Many tumors of this type offer meager clinical evidence of their presence, and symptoms may be lacking or referable to other organs. Several case reports are given to illustrate this point. Serial roentgenograms, included in the report, demonstrate the difficulties of interpretation not only of early, but also of advanced lesions. The authors urge surgical exploration for diagnostic purposes in the absence of bronchoscopic findings, since roentgen examination cannot be relied on for differentiation between tumors, tuberculosis, lung abscess, and other pulmonary lesions.—E. E. S.

Bronchial Adenoma. Jackson, C. L., Konzelmann, F. W., and Norris, C. M. [Temple Univ., Philadelphia, Pa.] *J. Thoracic Surg.*, **14**:98-105. 1945.

The symptoms, x-ray findings, treatment, and results of therapy of 20 patients having bronchial adenoma are presented in tabular form. Three of the patients were treated by lobectomy or pneumonectomy: 1 died 4 days after operation and the others were well after $4\frac{1}{2}$ and $5\frac{1}{2}$ years, respectively. Seventeen received bronchoscopic measures (forceps-removal, electrocoagulation) and, of these, 4 patients had x-ray or radon seed treatment in addition; they showed symptomatic improvement. There is detailed discussion of the pathologic features on which the diagnosis was based. The authors do not believe that malignant transformation of these tumors is common.—E. E. S.

The Problem of the So-Called Bronchial Adenoma. Graham, E. A., and Womack, N. A. [Washington Univ. Sch. of Med., and Barnes Hosp., St. Louis, Mo.] *J. Thoracic Surg.*, **14**:106-119. 1945.

The authors would prefer to designate these growths as mixed tumors rather than as adenomas because of the frequent presence of cartilage or even bone and sometimes of tumor formation by connective tissue elements. The possibility of malignant change is stressed, and pneumonectomy is urged as the procedure of choice. Several illustrative case histories are presented, with photomicrographs.—E. E. S.

Bronchial Adenoma Treated by Pulmonary Resection. Chamberlain, J. M., and Gordon, J. [Hosp. for Incipient Tuberc., Ray Brook, N. Y., and Home Folks Tuberc. Hosp., Oneonta, N. Y.] *J. Thoracic Surg.*, **14**:144-159. 1945.

This therapeutic measure is thought preferable to endobronchial removal because (a) it offers a definitive cure, (b) the hazards associated with the damaged lung distal to the tumor are avoided, and (c) some of these tumors are thought to be potentially malignant. Ten case reports are given. In each instance the extrabronchial portion of the tumor was larger than the part in the bronchus. There were no deaths following pulmonary resection; 2 patients died after bronchoscopy. In 5 cases lymph node involvement was encountered.—E. E. S.

Hamartoma (Often Called Chondroma) of the Lung. McDonald, J. R., Harrington, S. W., and Clagett, O. T. [Mayo Clin., Rochester, Minn.] *J. Thoracic Surg.*, **14**:128-143. 1945.

After a review of the reported cases of this abnormality and a discussion of terminology, 23 such lesions observed at surgery or necropsy at the Mayo Clinic are tabulated as to site, size, histologic appearance, and the age and sex of the patient. More detail is given concerning 3 cases in which the tumor was surgically removed. In no instance was there any suggestion of malignant transformation. Since, microscopically, all the elements of the adult bronchus were occasionally admixed with the cartilage, the authors regard the tumors as malformations developing from the bronchial anlage rather than as pure chondromas.—E. E. S.

Gastrointestinal Tract

Oesophago-Gastric Carcinoma. Laird, R. C. *Canad. M. A. J.*, **52**:610-612. 1945.

A case report. An early diagnosis made it possible to remove the growth. The use of sulfathiazole and of penicillin was believed to be helpful.—M. E. H.

Pernicious Anemia and the Early Diagnosis of Tumors of the Stomach. Rigler, L. G., Kaplan, H. S., and Fink, D. L. [Univ. of Minnesota, Univ. Hosp., and Minneapolis Gen. Hosp., Minneapolis, Minn.] *J. A. M. A.*, **128**:426-432. 1945.

The present report is concerned with 211 patients with pernicious anemia on whom roentgen studies of the stomach after a barium meal were made. Carcinoma of the stomach was found in 8%, benign tumors of the stomach in 7.1%. Cases are presented illustrating the rapid change from a benign polyp to a cancer, the presence of benign and malignant tumors side by side, and the development from a small barely detectable lesion to an extensive inoperable carcinoma. The routine x-ray examination of patients with pernicious anemia is valuable in detecting coexisting cancer early.—M. E. H.

Discussion on the Pathology and Treatment of Carcinoma of the Colon. Maingot, R., Dukes, C. E., and Lloyd-Davies, O. V. *Proc. Roy. Soc. Med.*, **38**:377-384. 1945.

Improved operation procedures have, during recent years, made many noteworthy advances in the management of patients suffering from carcinoma of the colon. The inoperable case is fast becoming a rarity, and the life expectancy following adequate extirpation of the diseased colon is relatively good. Indeed the colon represents one of the most favorable sites for cure of carcinoma.

Some of the principal factors responsible for the encouraging advances in this field include earlier recognition by improved radiological technic, methods available for the adequate decompression of the intestine, and the

bactericidal and bacteriostatic effects of specific drugs such as penicillin and the sulfonamides, *e.g.*, Sulfasuxidine. The fundamental principles of treatment recommended are as follows: (a) Preliminary accurate diagnosis of the position of the tumor is made by barium enema. (b) A hemoglobin percentage of not less than 85 is essential prior to operation. (c) A course of 20 gm. Sulfasuxidine daily for 4 days is administered as a routine. (d) The operation is a modification of the Paul-Mikulicz procedure, which it is claimed almost entirely eliminates ileus and peritonitis. (e) In cases of obstruction, decompression is accomplished by means of a Miller-Abbott tube or an indwelling intestinal tube attached to a suction apparatus.

Comparison of 331 cases of colonic and 1,000 cases of rectal cancer indicates that in rectal cancer the ultimate prognosis after operation is clearly related to the histology of the primary tumor (prognoses in low-grade malignancy, *i.e.*, Broder's Grade I, being more favorable than in higher grades), that tumors of low-grade malignancy are relatively more common in the colon than in the rectum, and that venous and lymphatic spread is less commonly found in operative specimens of colon cancer than in those of rectal cancer. Hence, colon cancer offers a more favorable prognosis than rectal cancer from pathological considerations.—L. W. P.

Treatment of Carcinoma of the Colon. Coller, F. A., and Vaughan, H. H. [Univ. of Michigan Med. Sch., Ann Arbor, Mich.] *Ann. Surg.*, **121**:395-408. 1945.

Among 173 patients operated on for carcinoma of the colon, resection was performed in 64.7% without gross involvement beyond lymph nodes, resection was done in 19% with gross metastases, and a palliative procedure was done in 16.2%. Immediate results alone are considered. Of the patients with hope for cure, only 1 died. The resection mortality was 4.1% and the over-all mortality 7.5%. In general, resection for cancer of the right colon in two stages, transverse incision for both right and left colectomy, early mobilization of patients, delayed closure of the abdominal wound after open anastomosis, closure of colostomies without the use of crushing clamps, preoperative decompression in the presence of obstruction, the use of complementary cecostomy as a safeguard, and primary resection with end-to-end anastomosis whenever feasible are favored.—W. J. B.

Retroperitoneum

Report of a Case of Retroperitoneal Hemangioendothelioma. Snodgrass, T. J. [Pember-Nuzum Clin., Janesville, Wis.] *Surgery*, **15**:988-993. 1944.

A report of a case in a 51 year old woman, who was in apparent good health 14 months after removal of the tumor.—W. A. B.

Liver and Gall Bladder

An Unusual Case of Primary Carcinoma of the Liver Associated with Diabetes Mellitus, Pulmonary Tuberculosis and Tuberculous Empyema. Clayman, S. G. [North Dakota State Tuberc. Sanitorium, Sun Haven, N. Dak.] *Journal-Lancet*, **65**:144-145. 1945.

A case report. The diagnosis was made at autopsy. Symptoms referable to carcinoma of the liver were present only shortly before death though postmortem findings suggested that the carcinoma must have been present for at least a year.—M. E. H.

Calcified Hemangiomas of the Liver. Aspray, M. [Spokane, Washington] *Am. J. Roentgenol.*, **53**:446-453. 1945.

Hemangiomas of the liver are common benign tumors, seldom identified prior to discovery by the surgeon or pathologist. They rarely calcify or become large enough to cause trouble; however the same type of calcification as is seen in hemangioma of flat bones may occur. Certain of these lesions have been found to respond to irradiation therapy. A case is reported in which calcified hemangioma of the liver was tentatively diagnosed by x-ray, and demonstrated later at autopsy.—E. H. Q.

Primary Carcinoma of the Gallbladder. Finney, J. M. T., Jr., and Johnson, M. L. [Union Memorial Hosp., Baltimore, Md.] *Ann. Surg.*, **121**:425-431. 1945.

Eighteen cases of primary carcinoma were found among 1,192 operations performed on the gall bladder in 10 years. Although there was one survival of 25 months, no patient was cured. Because of difficulty in diagnosis, discouraging results of operation after the diagnosis is suspected, and high correlation of cholelithiasis and carcinoma of the gall bladder, extirpation of calculous gall bladders is strongly advocated. No evidence other than frequency of association is advanced for the statement that cholelithiasis must be accepted as an etiologic factor in carcinoma of the gall bladder.—W. J. B.

Bone and Bone Marrow

Nonosteogenic Fibroma. Report of Two Cases. Wilson, A. L. [Chicago, Ill.] Report to Chicago Roentgen Soc., Apr. 12, 1945. From abstr. in *Proc. Inst. Med. Chicago*, **15**:361. 1945.

In the first case, tissue from a lesion in the cortex of the femur showed a microscopic appearance similar to that described by Jaffe and Lichtenstein (*Am. J. Path.*, **18**:205. 1942) as nonosteogenic fibroma of bone.

The second case is of interest because of the location of the lesion in the eighth left rib in the posterior axillary line. Previous authors had not recorded any instances of nonosteogenic fibroma in other than long tubular bones, though appearance elsewhere has been postulated.—M. E. H.

Les diversités anatomo-cliniques et évolutives des tumeurs à myéloplaxes des os longs. [The Diversity in the Anatomical, Clinical, and Developmental Aspects of Giant-Cell Tumors of the Long Bones.] Delarue, J., and Denoix, P. [Cancer Inst., Paris, France] *Presse méd.*, **51**:587-588. 1943.

Short case reports. Fibrous types are successfully treated by surgical curettage; hemorrhagic and necrotic types often require amputation.—C. A.

Solitary Myeloma of the Frontal Bone. Schwartz, C. W. [St. Agnes Hosp., White Plains, N. Y.] *Am. J. Roentgenol.*, **53**:573-574. 1945.

Solitary myeloma is a rare type of tumor. A case is reported with radiograph and photomicrograph.—E. H. Q.

Proliferative Lesions in Multiple Myeloma with Special Reference to Those of the Spleen. The Origin of the Plasma Cell. Lowenhaupt, E. [Univ. of California Hosp., and Mt. Zion Hosp., San Francisco, Calif.] *Am. J. Path.*, **21**:171-185. 1945.

"A lesion of the reticulo-endothelial system in multiple myeloma is described, in particular as it is found in the spleen and lymph nodes. In the spleen this lesion consists of the intrasinusoidal proliferation of plasma cells from sinus lining. In lymph nodes plasma cells proliferate in the interfollicular tissue. Lymphoid structures remain intact in both these organs. The presence of a closed system of littoral cells in the spleen, in contrast to that of lymph nodes, is suggested as the explanation for the localization of plasma cells in relation to sinus lining only in the former organ. These lesions, as well as the distribution of leukemic infiltration when the liver is involved, and the tendency to involve bone, suggest that plasma cells do not arise from lymphocytes or their immediate precursors, but that they arise, at least in this disease, from tissue histiocytes. It thus appears that plasma cell myeloma is more closely related to diseases of monocytic or clasmatocytic type than of lymphoid type. It is pointed out that the disease, multiple myeloma, at necropsy consists of a diffuse proliferative process involving the entire reticulo-endothelial system, regardless of its predominant skeletal or local onset." Clinical and pathological findings in 12 cases of multiple myeloma are presented.—J. G. K.

Plasma Cell Invasion of Peripheral Blood in Multiple Myeloma. Rubinstein, M. A. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **12**:616-623. 1945.

A case of multiple myeloma is presented in which the diagnosis was first suggested by the presence of plasma cells in the routine blood smear examination. The diagnosis was confirmed by bone marrow studies, which showed similar plasma cells. Subsequently, massive plasma cell invasion of the peripheral blood occurred. This resulted in the picture of plasma cell leukemia. The relationship of plasma cell leukemia to multiple myeloma is discussed.—Author's summary. (A. Cnl.)

Multiple Myeloma in a Youth. Wood, H., Quinlan, J. W., and Merrill, E. F. [U. S. Nav. Hosp., Newport, R. I.] *Am. J. Roentgenol.*, **53**:466-469. 1945.

A case is reported of multiple myeloma in a 19 year old male, with radiographs and a photomicrograph. The patient's general condition has improved under x-ray therapy, but the disease has remained uncontrolled.—E. H. Q.

Cystic Pelvic Chordoma Simulating an Ovarian Cyst. Reich, W. J., and Nechtow, M. J. [Cook Co. Hosp., and Cook Co. Grad. Sch. of Med., Chicago, Ill.] *Am. J. Obst. & Gynec.*, **49**:265-268. 1945.

A case report. A chordoma located in the pelvis was attached to the site of origin, lower thoracic vertebrae, by a long pedicle. Its location in the pelvis made it seem like an ovarian cyst.—A. K.

Muscle and Tendon

Myoblastoma. Howe, C. W., and Warren, S. [Pondville Hosp., Walpole, and Massachusetts Dept. of Pub. Health, and Harvard Cancer Commission, Boston, Mass.] *Surgery*, **16**:319-347. 1944.

Ten cases of this rare tumor are presented, and an analysis is given of 148 additional cases from the literature. More than one third of these tumors occurred in the tongue, and 56 were in the upper gastrointestinal or respiratory tracts. Eleven per cent of the total number of tumors were malignant. The tumor cell is derived from embryonic myoblasts, the typical cell being polyhedral with a finely granular cytoplasm. Striation may be present but is infrequent. Surgical excision, electrocoagulation, and radium therapy have all been used in the cases presented, but it is too early to evaluate their respective results.—W. A. B.

Leukemia, Lymphosarcoma, Hodgkin's Disease

The Leukemias. Miller, F. R., and Turner, D. L. [Jefferson Med. Coll., Philadelphia, Pa.] *M. Clin. North America*, 1376-1385. 1944.

A review in which infective, neoplastic, and metabolic, especially hormonal, theories of origin, as well as diagnosis and treatment, are discussed. The authors summarize their observations, published elsewhere, on the 2 specific substances, stimulating myeloid and lymphoid cells, that they have extracted from the urine of patients with various types of leukemia, lymphosarcoma, or Hodgkin's disease, and from the lipoidal fraction of normal beef liver.—J. L. M.

Involvement of the Genito-Urinary Tract in Leukemia. With the Report of a Case of Involvement of the Urinary Bladder. Pentecost, C. L., and Pizzolato, P. [Sch. of Med., Louisiana State Univ., and Charity Hosp., New Orleans, La.] *J. Urol.*, **53**:725-731. 1945.

Involvement of the kidneys in leukemia is fairly frequent, but bladder infiltration is rare. In the rapidly fatal case here reported, the first symptoms were from the bladder.—V. F. M.

Myelogenous Leukemia and Pregnancy. (A Report of Two Cases.) Miles, F. T., and Wheeler, D. [St. Boniface Hosp., St. Boniface, Canada] *Canad. M. A. J.*, **52**: 407-408. 1945.

The 2 cases are reported because of the development of myelogenous leukemia following earlier pregnancies. Both patients survived and had living babies; 1 patient was followed through two pregnancies, twins being born at the first; in both patients pregnancies occurred after the institution of radiation therapy for leukemia.—M. E. H.

Observations on Over One Hundred Cases of Myelogenous and Lymphatic Leukemia. Friedmann, A. B., and Meyer, L. M. [Kings Co. Hosp., Brooklyn, N. Y.] *Radiology*, **44**:341-343. 1945.

A series of 105 cases of myelogenous and lymphatic leukemia is reviewed. Differential diagnosis from other reticuloendothelial neoplasias should be made by blood and sternal puncture studies. Treatment is in the form of transfusions, drugs, and radiation. Radiation is given to the spleen, lymph node masses, or long bones, or by spray

irradiation to the whole body. With no pressure symptoms in moderately active cases radiation of long bones is advised.—R. E. S.

Changes of the Temporal Bone in Leukemia and Osteitis Fibrosa. Brunner, H. [Univ. of Illinois Coll. of Med., Chicago, Ill.] *Arch. Otolaryng.*, **39**:1-13. 1944.

The temporal bone was taken for careful microscopic study at the postmortem examination of a 62 year old woman who had had chronic lymphatic leukemia. Leukemic infiltrations were present in the majority of the marrow spaces within the periosteal layer. Bone trabeculae within the marrow were often covered with osteoid substance, indicating bony deposition. Lacunar resorption was observed. The endosteal and enchondral layers of bone were not involved by leukemic infiltration; the involvement of the periosteum alone indicates that the disease process may have started any time within the fetal period or in adult life.

Temporal bone changes in a patient with osteitis fibrosa are also described, and the relation between that illness and Paget's disease is discussed, with a review of the literature.—W. A. B.

Acute Plasma Cell Leukemia. Meyer, L. M., Halpern, J., and Ogden, F. N. [Kings Co. Hosp., Brooklyn, N. Y.] *Ann. Int. Med.*, **22**:585-598. 1945.

Case report, with photomicrographs, discussion, and a review of the literature with 43 references.—J. G. K.

Hodgkin's Disease. Isaacs, R. [Michael Reese Hosp., Chicago, Ill.] *M. Clin. North America*, 201-213. 1944.

A review of Hodgkin's disease in which the following topics are discussed: terminology, incidence, diagnosis, clinical symptoms, prognosis, and treatment. Two cases are presented to illustrate a benign and a malignant type, and to emphasize certain features in diagnosis and treatment.—J. L. M.

The Relation of Hodgkin's Disease, Lymphosarcoma and Reticulum Cell Sarcoma. Herbut, P. A., Miller, F. R., and Erf, L. A. [Jefferson Med. Coll., Philadelphia, Pa.] *Am. J. Path.*, **21**:233-253. 1945.

Six cases are presented that at one time during the lives of the patients were diagnosed as Hodgkin's disease and at another time as lymphosarcoma and that at autopsy showed various combinations of Hodgkin's disease, lymphosarcoma, and reticulum cell sarcoma. The authors explain these facts by considering that the three diseases arise from a common stem cell—the reticulum cell—and then differentiate in one direction or another according to the amount and type of stimulation provided by certain "specific hemopoietic stimulators," as described in experimental work published elsewhere. According to this view, proliferation arising because of an excess of the lymphoid stimulator gives rise to a lymphosarcoma, while that due to an excess of both lymphoid and myeloid stimulators gives rise to Hodgkin's disease provided the stimulation is accompanied by maturation, or to reticulum cell sarcoma when unaccompanied by maturation; combinations result when the specific stimulators are not uniformly distributed throughout the organs or when a temporary excess of one is followed by a temporary excess of the other.—J. G. K.

Adrenal

Adrenal Cortical Tumors—Physiologic Considerations. Kenyon, A. T. [University of Chicago, Chicago, Ill.] *Surgery*, **16**:194-232. 1944.

An outline of the recent work bearing on adrenal hyperfunction and adrenal cortical steroids, with a discussion of the physiologic mechanisms explaining some of the symptoms of adrenal tumors. The bibliography contains 141 references.—W. A. B.

Hormonal Tumors of the Adrenal. Cahill, G. F. [Columbia-Presbyterian Hosp., New York, N. Y.] *Surgery*, **16**: 233-265. 1944.

A summary of the recognized clinical effects and means of diagnosis of tumors of the adrenal cortex, and of hormone-producing tumors of the adrenal medulla. Several cases from the Presbyterian Hospital, some previously reported, are cited.—W. A. B.

Pituitary

The Endocrine Effects of Pituitary Tumors. A Clinical Review. German, W. J. [Yale Univ. Sch. of Med., New Haven, Conn.] *Surgery*, **16**:47-81. 1944.

The original clinical descriptions of the various syndromes associated with pituitary tumors are quoted, and photographs of patients are reproduced. The endocrine effects in acromegaly, chromophobe adenoma, Simmonds's disease, mixed adenoma, and basophilic adenoma are tabulated with percentage incidence, from previously reported series of cases, and postmortem findings are reviewed. Ninety-five references are included in the bibliography.—W. A. B.

Cerebrospinal Rhinorrhea Associated with Craniopharyngioma and Meningitis. Berger, O. [Harlem Hosp., New York, N. Y.] *Arch. Otolaryng.*, **39**:184-185. 1944.

A case report.—W. A. B.

Craniopharyngeoma and Suprasellar Adamantinoma. Globus, J. H., and Gang, K. M. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **12**:220-276. 1945.

Fourteen cases of hypophyseal duct tumor are described. The tumors often implicated the third ventricle, thereby causing hypothalamic disorders. Clinically, these growths manifested themselves by vegetative disturbances, signs of increased intracranial tension, signs due to direct pressure by the tumor, and x-ray findings consisting of calcifications in or about the sella turcica. Surgical intervention in several instances resulted in but temporary relief and at best in a relatively longer survival period; 6 patients died in the immediate postoperative period.—A. Cnl.

Thyroid and Parathyroid

Symposium on Surgical Lesions of the Thyroid. Goiter in Children. Pemberton, J. de J., and Black, B. M. [Mayo Clin., Rochester, Minn.] *Surgery*, **16**:756-763. 1944.

A review of cases of goiter in children, seen at the Mayo Clinic from 1908 to 1943, shows 189 patients under 14 years with exophthalmic goiter, 52 with adenomatous

goiter, and 53 with nodular goiter. Of the nodular goiters, 18 showed carcinoma; 13 of these were adenocarcinomas of the papillary type, and metastases had developed in 11 cases.—W. A. B.

The Endocrine Activity of Thyroid Tumors and the Influence of the Thyroid Hormone on Tumors in General. LERMAN, J. [Massachusetts Gen. Hosp., Boston, Mass.] *Surgery,* **16**:266-272. 1944.

A review of the literature.—W. A. B.

The Endocrine Aspect of Enlargements of the Parathyroid Glands. COPE, O. [Harvard Med. Sch., and Massachusetts Gen. Hosp., Boston, Mass.] *Surgery,* **16**:273-288. 1944.

In the Massachusetts General Hospital series of 78 cases of primary hyperparathyroidism, 71 were caused by parathyroid adenoma, and the diagnosis was suggested by renal tract calcification in 43 of the patients. The differentiation between primary and secondary hyperparathyroidism may be difficult.—W. A. B.

Hyperparathyroidism. Report of a Case. ROTH, H. S. [New York, N. Y.] *J. Mt. Sinai Hosp.,* **12**:598-606. 1945.

A case of hyperparathyroidism, in which the signs and symptoms were almost entirely urologic in character, is reported. At operation, a large cystic parathyroid adenoma, which had been suspected preoperatively because of clinical and roentgenographic evidence, was found and removed. Four months afterward, renal function had returned to normal.—A. Cnl.

STATISTICS

L'âge du cancer. [The Cancer Age.] HUGUENIN, R., and BERTHON, J. [Cancer Inst., Paris, France] *Presse méd.,* **50**:610-611. 1942.

Cancer may develop at any age, but the highest incidence is found between the ages of 35 and 55. Statistics based on 9,620 cases are given.—C. A.

A propos de l'âge du cancer. [Apropos of the Cancer Age.] LUMIÈRE, A. *Presse méd.,* **51**:62-63. 1943.

Cancer statistics compared to vital statistics clearly indicate that the incidence of cancer progresses with age and that, contrary to the assertion of Huguenin and Berthon [see preceding abstract], cancer is not more frequent at 40 than at 80.—C. A.

La fréquence des cancers. [The Incidence of Cancers.] HUGUENIN, R., and BERTHON, J. [Cancer Inst., Paris, France] *Presse méd.,* **50**:723-724. 1942.

A statistical study of the incidence of the different types of human cancer at various ages, and the death rate from cancer in France during the 1930's—C. A.

The Statistical Approach to the Cancer Problem in Massachusetts. POTTER, E. A., and TULLY, M. R. [State Dept. of Pub. Health, Boston, Mass.] *Am. J. Pub. Health,* **35**:485-490. 1945.

Material was compiled to demonstrate the integration of statistics in the Massachusetts Cancer Program. A few examples were chosen to show different types of studies and some of the different approaches. The findings in some of the studies influenced the program as a whole; in others only in part. The decrease in the death rate and the reduction in delay before seeking diagnosis were measures of the efficacy of the program. The statistical approach is fundamental in attacking the cancer problem from the public health standpoint.—M. E. H.

Cancer in Kansas. BEELMAN, F. C. [Kansas State Board of Health, Topeka, Kans.] *J. Kansas M. Soc.,* **46**:145-149. 1945.

A statistical analysis of 50,393 cancer deaths, based on annual reports since 1916. Although cancer is a notifiable disease in Kansas, less than 25% of the cases are reported.—M. L.

CANCER CONTROL AND PUBLIC HEALTH

Quelques données sur la cancérose. [Some Facts on Cancer.] GOFFIN, R. [Brussels, Belgium] *Presse méd.,* **53**:339-340. 1945.

A report on the anticancer campaign in the Belgian Mutual Socialist Societies.—C. A.

Treatment of Cancer. Directions for the Use of Record Cards. MINISTRY OF HEALTH. Circular (March 1945).

The Ministry of Health (Whitehall, London S.W.1.) in consultation with the Radium Commission (12, Manchester Square, London, W.1.) has produced cards for recording the treatment and follow-up of cancer patients under the Cancer Act 1939, and a booklet of instructions for filling up these forms.

"The data to be recorded have been reduced to the simplest practical form for obtaining information . . . ." but "it is hoped that some hospitals will be able to undertake more detailed studies. . . . ." "The term 'Cancer' comprises:

> Malignant neoplasms of all kinds, including carcinoma; sarcoma; astrocytoma; blastoma (with or without prefix); chloroma; chordoma; endothelioma; ependymoma; epithelioma; Ewing's tumour; glioma (unless stated to be benign); malignant cachexia or disease; malignant reticulosis, including Hodgkin's disease; melanoma; myeloma; papilloma choroideum; pinealoma; rodent ulcer; scirrhus; seminoma; leukaemia. 'Malignant' includes invasiveness with or without metastases."

A Registration Card and an Abstract Card are required for each patient, and if a second primary growth appears fresh cards must be added to these. All Abstract Cards relating to patients registered within a given calendar year are to be sent to a National Statistical Bureau after the entry for the first yearly follow-up has been made. Thus cards of patients treated in 1945 would be sent in 1947. Abstract Cards of patients who died during the year in question, or in whom the provisional diagnosis of malignant disease was not confirmed, will be retained by the Statistical Bureau. Abstract Cards of patients still living are to be sent to the Statistical Bureau again in the third and fifth years. The amount of writing is lessened by drawing a circle round the number following the term which is applicable. Definitions of "early" new-growth in the breast, cervix uteri, mouth, and skin are given in the booklet. "Main Treatment" is to be described as surgery, radium, radon, radium beam, low voltage (under 170 kv.), or high voltage x-ray.—E. L. K.

[See following page.]

Reproduction of Both Sides of Abstract Card Required by British Ministry of Health [see preceding abstract]

CASE ABSTRACT CARD ..... **CENTRE**

C National Registration Identity No. ..... **AREA ORGANISATION**

Registration No. ..... Hospital No. .....

Surname ..... Age ..... Sex .....

Christian Names in full .....

Registration Date .....

Provisional Diagnosis on Registration Card : .....

.....

First Sign or Symptom (with approximate date) .....

.....

If not treated at place of registration, state: Too Advanced 1 Refusal 2; Concurrent Disease .....; Referred to .....

If found Non-Malignant, state Final Diagnosis with M.R.C. Code No.:

*(198) Wt. 58892/7283 50M 6/45 S.E.R. Ltd. Gp. 662.

Diagnosis : (site and nature of Primary Growth) .....

.....

M.R.C. Code No. .....

If previously treated elsewhere, state method None 1 Surgery 2 Radiotherapy 3 Surgery+R.T. 4 Other Methods 5

Treatment for relief of symptoms only

.....

Healed 1 Residual 2 Recurrent 3 Metastatic 4

Clinical Findings :

Primary Growth : None 1 Early 2 Late 3

Secondary Nodes : None 1 Mobile 2 Fixed 4

Other Metastases : None 1 One Area 3 More than one area 5

Present (name Sites)

Histology :

At time of treatment :

By Biopsy 1 Malignant 4

,, Whole Tumour 2 Non-malignant 5

,, P.M. 3 Indeterminate 6

None 7

Subsequent correction (if any) : Date of correction .....

Biopsy 1 Malignant 4

Whole Tumour 2 Non-malignant 5

P.M. 3 Indeterminate 6

(Reverse side of card)

TREATMENT

Date

Intention : Radical 1 Palliative 2

MAIN TREATMENT

Organ or Region — Operation or Method

Other Treatment

FOLLOW-UP

Years after First Main Treatment	Date of Examination (E) or Report (R)	Alive: Primary Growth Present	Alive: Metastases Present	Alive: No Evidence of Growth	Alive: Clinical Findings to Date Indeterminate	Died: Primary Growth Present	Died: Metastases Present	Died: No Evidence of Growth	Died: No Information as to Persistence of Growth	Not Traced
0–										
1–										
2–										
3–										
4–										
5–										
7–										
10–										
15–										
20–										

Date of Death :

Radical Treatment Completed 1

Palliative Treatment Completed 2

Treatment Not Completed 3

Post Mortem Findings :